INHERITANCE OF CALLOSITIES IN THE OSTRICH

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"The problem of the method of evolution is one which the biologist finds it impossible to leave alone, although the longer he works at it, the farther its solution fades into the distance. The central point in the problem is the appearance, nature, and origin of the heritable varieties that arise in organisms."—H. S. JENNINGS.[2]

THE ostrich has a shield-like sternum devoid of a keel, a character it shares with the rest of the Ratitæ. The middle forms a broad, rounded projection, while the covering skin is greatly thickened, devoid of feathers, and constitutes a large, dense callosity on which the bird rests when crouching. Moreover, the ostrich is unique among birds in having a symphysis pubis, which forms a ventral projection behind corresponding with the one in front, only smaller, the skin over it likewise showing a strong callosity (Fig. 1). The result is that when the bird crouches the two median projections come into direct contact with the ground and the thickened pads support the greater part of the weight of the body, about 250 lbs., in front and behind, while it is steadied laterally by resting upon the upper surface of the nearly horizontal meta-

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[1] The author is indebted to Dr. Raymond Pearl for seeing the paper through the press.

[2] *Journ. Washington Academy of Sciences,* Vol. VII, No. 10, May 19, 1917, p. 281.

tarsals and feet (Fig. 2). The sternal and pubic callosities may therefore be looked upon as a direct response of the skin to the pressure and friction of the body against the hard ground. Also in its frequent habit of
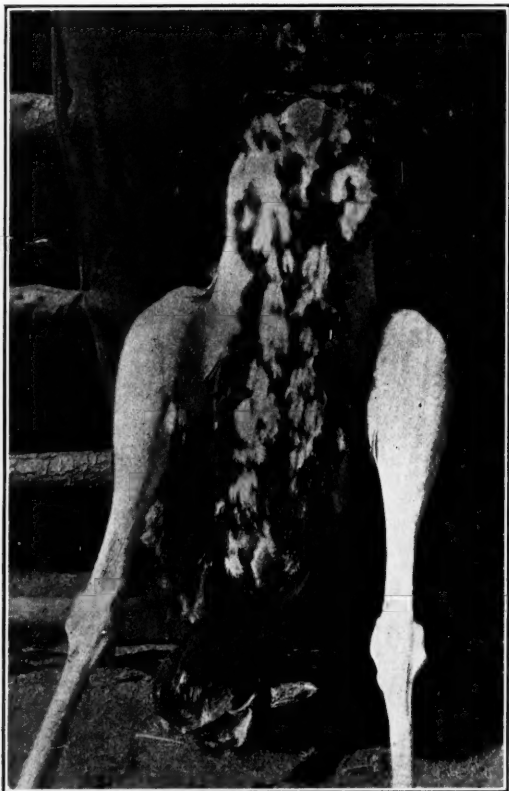

FIG. 1. Under surface of ostrich showing the large sternal callosity in front and the small pubic callosity behind. The darkened surface of both is due to the adherence of dirt. The bird is a young cock about eighteen months old in which the white ventral feathers are not yet completely replaced by black.

taking a "dust-bath" the ostrich rolls from side to side, the two projections being in the axis of motion, and this serves further to extend the area subject to pressure and friction.

In man and mammals generally a callosity usually con-

sists of a single, smooth or papillose thickened area of
the skin, resting upon a bony support; but in the ostrich,
as in other birds and in reptiles, it is constituted of a
number of separate and distinct thickenings, somewhat
regular in their arrangement, which give the appearance
of a rounded or angular mosaic or tessellation (Figs. 5
and 6). This is typically shown on the under surface of
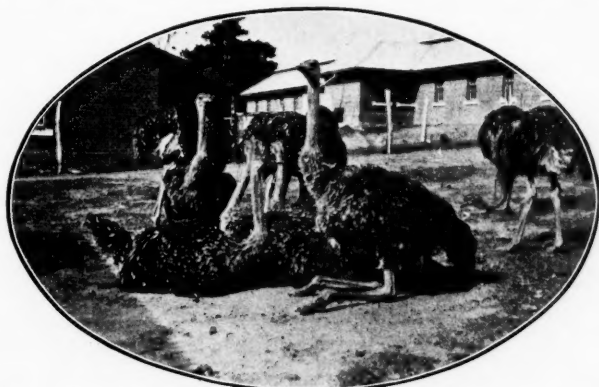the toes of birds and lizards, where the elements tend to

FIG. 2.   Group of young ostriches, about six months old, the one in the fore-
ground seen in a half-crouching attitude. The weight of the body is supported
upon the inside of the ankle and the partly upturned two toes. When fully
crouching the bird lurches forwards and comes to rest upon the sternal and pubic
callosities, the tarsus and toes remaining in the same position.

be elongated and present a coarsely villous effect. Where
the skin is scaly each callous constituent corresponds
with an individual scale, but the latter has evidently no
determining influence upon the form assumed, for the
same tessellated arrangement is found over the sternal
and pubic thickenings, though no scales are present. It
is probable that the typical form of the reptilian callosity
was first determined by the presence of the epidermal
scales of the skin, and the latter still responds in the
same manner in birds, not only on the legs and toes where
scales occur, but over other parts of the body from which
they are absent. The present interest lies in the fact that
the characteristic form assumed by a callous area in the
ostrich enables it to be sharply distinguished from the

surrounding parts of the skin which remain smooth. The tessellation, along with the thickening below, gives it a distinctive character as compared with the pads in mammals, which are mere thickening of the skin, and whose claim to be regarded as a "character" might at times be disputed. Where a callosity assumes any considerable thickness the underlying bone exhibits a correlated response by likewise becoming thickened, as is well shown on both the sternum and pubis of the ostrich.

The skin of all vertebrates appears to have the inherent power of responding to frequently repeated pressure and friction by the formation of thickenings over the bony projections upon which it rests. The pads are special protective adaptations to meet intermittent pressure and friction, upon what would otherwise be soft vulnerable parts of the body. They can arise at any part of the surface of the skin and may slowly disappear when the causal stimuli are no longer operative. Many of them are temporary responses, acquired during a part of the life-time of the individual, and come under the group of adaptive somatic modifications which are non-transmissible, though others, especially those on the under-surface of the feet, are transmissible and may therefore be regarded as germinal in their origin. *Thus similar characters, alike in structure and function, may be either individually acquired and non-transmissible or germinal and heritable.*

The ostrich resembles man and other animals in having the inherent power to produce special callosities over parts of the skin not usually subjected to pressure and friction, as the following observation proves. A chick was hatched in the incubator with its legs widely apart, in such a manner as to be incapable of supporting itself upright in the normal fashion. A deformity of this nature is not unusual among both ostrich and poultry chicks as a result of imperfect incubation, but can generally be rectified by bandaging the legs and drawing them nearer together for a day or two. In this instance however advantage was taken of the deformity to deter-

mine how far the skin would respond to unusual friction and pressure. With its legs widely apart, the chick naturally lay almost prone upon the ground, the inner side of the ankle constituting a feeble support, the tarso-metatarsus having here a projecting knob. The chick was able to raise itself slightly upon the latter and also to drag itself along the ground. It was kept alive for about ten days, and in that time developed a very conspicuous callous thickening over the inside of the metatarsal knob just below the ankle, the normal hereditary callosity along the back of the ankle being unused. The thickening was covered with the minute scales present over the leg generally, but the degree of friction was too intense and continuous for the skin wholly to adapt itself, and a slight abrasion occurred at the apex of the thickening, as in the human hand where pressure and friction are applied too continuously for the callous formation to keep pace with them.

The sternal and pubic callosities are not the only ones in the ostrich which appear to represent adaptive responses to the special habits of the bird. When taking its frequent sand-baths, it rolls about in the dry sand or dust, from side to side, and at the same time uses its wings in an oar-like manner. During the process the under surface of the latter is dragged over the ground and then turned upwards, inwards and backwards, scattering the sand or dust over the body generally, first from one wing and then from the other. The front or pre-axial border of the wing is necessarily subjected to much friction, and develops slight callous areas wherever the internal bones project. Further, the third digit of the wing, which is usually buried in the flesh, is occasionally found projecting freely from the under surface, and its tip naturally comes in for a good deal of rough wear as the latter is dragged along the ground. In response, it becomes knob-like and thickened, the surface showing the characteristic callous markings (Fig. 3). The free tip of the supporting phalanx is also knobbed.

Taking into account the responsive nature of the skin

along with the activities of the ostrich there appears no reason why the sternal, pubic and alar callosities should not be regarded as direct, structural responses to the pressure and friction to which these parts of the body are subject in the every-day activities of the bird. They could be understood as acquired, adaptive characters. The experiment given has served to prove, what would naturally be expected from experience with other animals, that the skin generally is endowed with the power
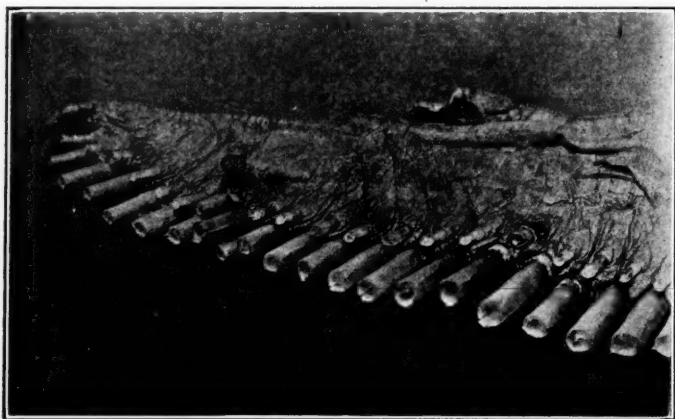

Fig. 3. Under surface of wing showing projecting third digit. The clawed ala spuria is seen above, the second finger is axial and also clawed, while the third projects freely from the under surface and is callous and knobbed.

to make callous responses when subjected to the necessary stimuli.

It was with some surprise therefore that in a series of embryos, representing all the stages passed through during the 42 days of incubation of the ostrich, the later ones were found to possess a perfectly developed callosity over both the sternum and the pubis, of exactly the same form and nature as in the young chick and adult (Fig. 4). The papillary outlines shown to be such a characteristic feature of sauropsidan callosities have the same variations in size and distribution as in the adult, and serve clearly to delimit the callous area from the remaining smooth

surface of the body. Examination of chicks from the
time of hatching onwards leaves no doubt that the pre-
natal callosities become those of the adult, the elevations
becoming larger and coarser with use.

The rather insignificant callosities on the wing also
show themselves on unhatched and newly hatched chicks.

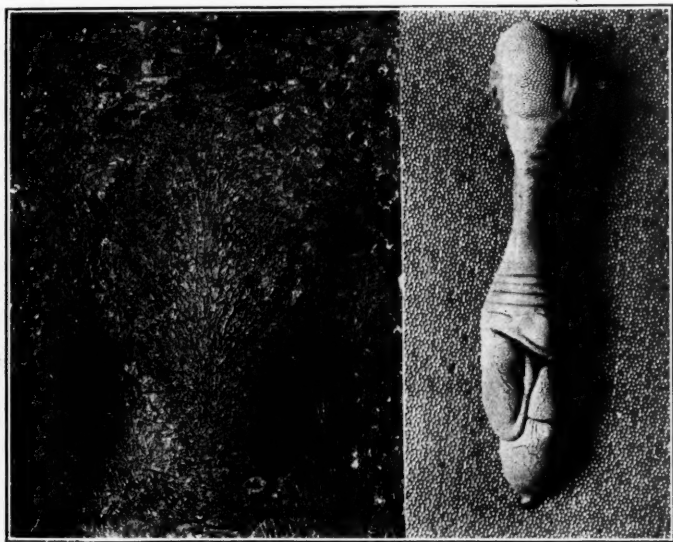4   5

FIG. 4.   Sternal covering of ostrich chick two or three weeks after hatching,
showing the hereditary callosity fully established and functional. The cut ends
of the feathers are seen surrounding the naked area.

FIG. 5.   Callosities on foot and ankle of ostrich chick a few days before hatch-
ing. The thickenings are already well developed, the separate elevations on the
toes being much narrower and closer than those on the ankle.

They are hardly distinguishable by any special thicken-
ing of the skin, but by the appearance of a faint reticula-
tion in places corresponding with those in which they are
found in the adults, and which serves clearly to separate
them from the surrounding smooth surface.   Even the
tip of the third digit where sufficiently projecting shows
a few markings, leaving no doubt they would later become
the functional callosity.

We have therefore in the ostrich certain hereditary structural characters whose independent formation could in every respect be accounted for during the life-time of the bird from the known responsive nature of the skin and the habits of the creature. Examination of the adult alone and a knowledge of its activities would have justified us in regarding them as acquired adaptive characters, had not observation proved that they appear on the chick prior to hatching, and before the parts could have been subjected to the usual stimuli. *The ostrich has hereditary characters which could also be produced as adaptive responses to the habits of the bird.*

The old contentious question therefore arises as to whether the character first appeared as a response of the skin to the habits of the ostrich and has now become hereditary, or whether, having arisen fortuitously in the germ plasm, wholly apart from any adaptive need of the bird, it is now utilized by it. Has the habit developed the character until it has become transmissible or, the character being given, has it permitted of the adoption of the habit? The reply is simple and free from doubt: the callosity under any circumstances would develop *pari passu* with the habit and need of the bird, and neither the callosity nor the habit is dependent upon any antecedent formation. If the character did not arise in the first instance from the activities of the bird, subsequently becoming transmissible, it is manifest that it could originate by two distinct and independent methods, namely, from the germ-plasm and from post-natal stimuli.

It is not the first time that the presence of callosities in the embryos of animals provided with them in the adult has been adduced as evidence that characters originating during the life-time may be transmitted to the offspring. The best known case is that of the wart-hog, another African type—*Ex Africa semper aliquid novi.* With reference to this Professor J. Arthur Thomson[3] remarks:

[3] "Heredity," London, 1912, p. 180.

The African wart-hog (*Phacochærus*) has the peculiar habit of kneeling down on its fore-limbs as it routs with its huge tusks in the ground and pushes itself forward with its hind-limbs. It has strong horny callosities protecting the surfaces on which it kneels, and these are seen even in the embryos. This seems to some naturalists to be a satisfactory proof of the inheritance of an acquired character. It is to others simply an instance of an adaptive peculiarity of germinal origin wrought out by natural selection.

In the latter part of the above quotation Thomson merely presents the two opposing views without affording us the advantage of his own. The last sentence is a succinct expression of present-day orthodoxy, and we may well consider how far it is justifiable in the case of the ostrich. It is manifest that from their very nature the callosities are outside the realm of competitive strife, and therefore could not have been "wrought out by natural selection." If a character is such that it must perforce be produced as a result of the every-day activities of an animal it is as wholly gratuitous to invoke natural selection as it would be to seek an independent germinal origin. As already shown, the skin of the ostrich is of such a nature that it will form callosities wherever friction and pressure are intermittently applied, just as surely as they will be produced on the human hand as a result of manual labor, on the finger tips of the harpist, violinist or rosary devotee, or on toes encased in ill-fitting boots, with all of which natural selection has no concern. Originally natural selection may have been operative in the survival of animals having the inherent power to form the thickenings, but we have abundant evidence that all the higher forms now possess it.

When the ancestral ostrich first took to resting on its sternum and pubis and rocking from side to side, the callous thickenings would arise quite apart from any antecedent formation and whether or not the germ-plasm had anticipated the need. An inherent power is transmitted, and nothing is gained by transmitting the callosities themselves, since they are adaptations which could arise in the natural course as needed. No selection

is involved in producing the "horny hand of toil"; it forms in the individual in proportion to the need for it. If fore-doomed to hard manual labor some advantage may possibly be conceived in having the callosities in advance, but would be insufficient to be of any selection value.

The position resolves itself as follows: From the known responsiveness of the skin of the ostrich to intermittent pressure and friction and the established activities of the bird it is just as certain that the sternal, pubic and alar callosities could be acquired in each generation independently as that similar thickenings could develop on the palm of the human hand engaged in labor. If we are not prepared to admit that the callosities first arose as somatic adaptations and then became hereditary, we have to face the alternative that at some time in the history of the ostrich a change took place in its germ plasm of such a nature as to give rise to a directly adaptive character, altogether similar to what could be somatically acquired; we have to admit that an exactly similar character could be produced in two wholly different ways: (*a*) directly as a response to the activities of the bird; (*b*) as a result of germinal changes. The same character could be somatically acquired and could arise germinally.

Of course the same argument could be applied to the strongly marked callosities on the toes and ankle of the ostrich which are also hereditary (Fig. 5). But these are not so peculiarly specific for the present purpose. Hereditary pedal thickenings occur in most animals, and even Darwin[4] regarded the thickened sole of unborn infants as "the inherited effects of pressure during a long series of generations." The thickenings on the sternum, pubis and wings are confined to the ostrich, and therefore afford a more circumscribed case for discussion, hereditary transmission from any other type being placed out of consideration, though it is not unlikely that some of the other Ratites may have corresponding structures.

An acquired, non-transmissible, callous pad, presumably due to a change in the crouching habit of the ostrich

4 "Descent of Man," p. 18.

in the course of its phylogeny, remains to be noticed, as a
further instance of the responsive power of the skin.
Near the mesotarsal ankle-joint occurs a strong, elon-
gated, hereditary callosity covering the median part of
the broad, proximal end of the tarso-metatarsus (Fig.
5). This pad would naturally be used if the bird rested

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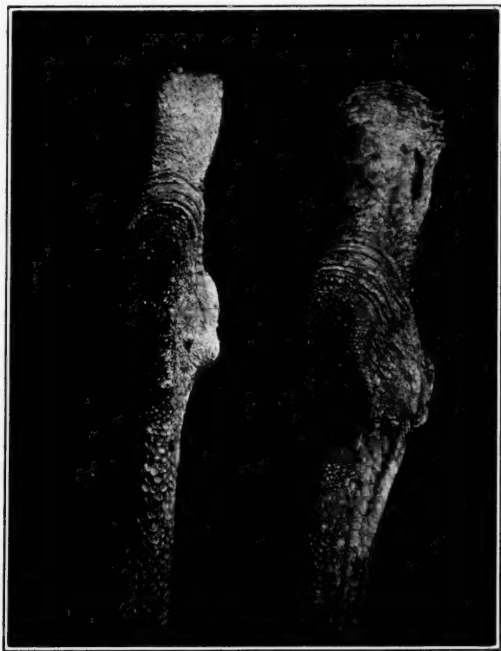
FIG. 6. Ankle region of young ostrich showing the symmetrical hereditary
callosity above and the accessory one forming below on the inside (to the left).

FIG. 7. Ankle region of old ostrich in which the accessory ankle callosity (on
the right) has become coarse and broken up.

squarely upon its tarsus and foot when crouching, the
weight being mainly on the ankle. The ostrich however
makes little or no use of it, for even in young chicks
scarcely any evidence of contact with the ground can be
observed. Somewhat to the inside there appears a new
callous thickening, which begins to form by the time the
chicks are a month or two old, and remains as the func-

tional pad throughout life, taking the place of the hered-
itary one which, though hardly used, persists structurally
(Figs. 6 and 7). The new callosity is continuous laterally
with the old median one, and altogether resembles it in
character. No trace of it however appears in the chick
prior to hatching (Fig. 5), hence it represents an indi-
vidually acquired, adaptive character in the truest sense.
We have therefore an original part of the ankle callosity
which is hereditary, though now non-functional, and an
acquired part which is functional and non-transmissible.[5]

The main facts presented seem capable of interpreta-
tion only in one of two ways: (*a*) *An acquired character
which represents a structural response to stimuli result-
ing from the activities of the organism may become
transmissible.* (*b*) *A character may arise germinally of
a form and nature exactly similar to one which would
otherwise be acquired independently from the known
activities of the organism and the established responsive
nature of its structural parts.*

[5] Manifestly in the course of its phylogeny some change has taken place
in the manner of crouching of the ostrich, for instead of resting squarely
upon the median part of the ankle it has come to support itself mainly upon
the inside. One ventures the suggestion that the change is to be associated
with the loss of the second toe in the course of the retrogressive evolution
of the foot. During a part of its phylogenetic history the ancestral African
ostrich had unquestionably three toes like the living *Rhea,* the American
three-toed ostrich, representing the second, third and fourth of the penta-
dactyle series. The second has disappeared in the two-toed ostrich *Struthio,*
though considerable traces exist in the embryo.

In its three-toed stage the ostrich would rest squarely upon its ankle, the
other extremity of the limb being steadied by the upturned three toes, a
smaller one on each side of the large middle third. A symmetrical median
callosity would naturally form at the ankle-joint and, according to the view
here maintained, would become transmissible. With the loss of the inner or
second toe through degeneration the inside distal support for the tarsus
would disappear, and the latter would tend to tilt inwardly along its whole
length, in such a manner that the median part of the ankle would no longer
support the bird. The callosity over it would be unnecessary, but a new
one would form over the new area of support. In the ostrich of to-day the
ancestral, hereditary ankle callosity, reminiscent of the three-toed stage, still
appears, though functionless; a new non-hereditary one is acquired afresh
with each generation and assumes the function of the old, becoming the
ankle support for the crouching two-toed bird (Fig. 7). The whole forms a
remarkable illustration of correlation between a phylogenetic change and an
adaptive ontogenetic modification.

In adopting the first interpretation we depart from the generally accepted opinion of biologists of the present day and admit that an acquired character may become transmissible; in maintaining the second we are exercising a credulity unjustified by biological experience.

In the voluminous literature of evolution and heredity, case after case has been brought forward by advocates such as Lamarck and Herbert Spencer, claiming to be illustrations of the inheritance of acquired characters, and just as surely has it seemed possible to interpret them in some other fashion, as Weismann and others have insistently done. The fate which has befallen these should suffice to make the boldest hesitate in adducing yet another. It is the apparently unassailable character of the two opposing statements above which emboldens one in all diffidence to re-open "the interminable question" of the late Professor W. K. Brooks, that leader and inspirer of so much American philosophical biology. The peculiar justification for the present claim seems to be that, were the callosities of the ostrich not transmissible, they could be acquired just as effectively from the responsive nature of the skin of the bird; also that natural selection has no bearing on the question, for they are adaptive structures which the organism has the inherent power to produce as required.

According to Weismann (quoted from Walter[6]) three things are necessary to prove the inheritance of acquired characters: "*first,* a particular somatic character must be called forth by a known external cause; *second,* it must be something new or different from what was already exhibited before, and not be simply the re-awakening of a latent germinal character; and *third,* the same particular character must reappear in succeeding generations in the absence of the original external cause which brought the character in question forth." It is contended that all the circumstances surrounding the sternal and pubic callosities of the ostrich are in full accord with these three requirements.

When assuming that an acquired character has become

[6] H. E. Walter, "Genetics," Macmillan & Co., 1913, p. 94.

transmissible it is usually held that in some mysterious fashion it has so impressed itself upon the soma that it becomes represented in the germ plasm by one or more factors, determinants or genes which are able to reproduce the same character in the next generation. The difficulties of conceiving this are so great as to convince most students of its impossibility. On the other hand we have to admit that we know little as to the means by which a germinal factor arises and gains its expression as a somatic character. Apart from the accessory chromosome in sex cells and the highly suggestive work of Professor T. H. Morgan and his associates on germinal *loci* in *Drosophila,* we only know of factorial representation by somatic expression. We are ignorant of the relationships between the two, and of the measures by which one gives rise to the other. Were it not that Thomson has shown the contrast to be hardly justifiable, one would be inclined to ask: Is it not as difficult to understand how a genetic factor arises and comes to have somatic expression as it is to conceive how germinal representation may be gained by an acquired somatic character? We accept the one without demur, but are prone to deny the other as impossible. We must not forget the warning of Professor Lloyd Morgan that because the phenomenon of acquired transmissibility can not be understood it is not necessarily rendered impossible.

In considering the difficulty in the way of an acquired character gaining factorial representation in the germ plasm it is legitimate to enquire whether a transmissible character is necessarily germinal as present-day teaching so consistently affirms, that is, whether it is necessarily represented in the germ plasm by definite genetic factors.[7] We have admitted that we know little or noth-

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[7] In a sense everything appearing in the soma may be regarded as derived from the germ, but the factorial hypothesis has given us a clear understanding as to what is meant when we say that a character is germinal. With the question of acquired characters before us there need be no confusion as regards a germinal and a non-germinal character, and whether the latter appears pre-natally or post-natally. On the considerations here set forth a transmissible character is not necessarily represented directly by germinal genetic factors.

ing of the manner in which factorial representation in the germ plasm gains expression in the soma; on the other hand we have some experience, from observation and experiment, of the production of somatic changes in the life-time of the organism, as a result of environmental influences and of stimuli due to the use and disuse of parts. The production of callosities, the variation of muscles and the skeletal changes in correlation there-with, the direct modification of bones, ligaments and mesenteries, are all adaptive changes which may result as responses to the external and internal stimuli to which the organism is subject during its life-time.[8] They reveal the inherent powers of responsive adaptability present in the tissues and organs of the body. They are in truth characters which arise independently of direct represen-tation in the germ plasm, and indicate that the latter is not the *fons et origo* of all structural changes. The power of the tissues to respond to stimuli is transmissible; ir-ritability, the power of responding to stimuli, is one of

[8] In a series of papers appearing in the *Journal of Anatomy and Physiology* from 1886 to 1888, Sir W. Arbuthnot Lane presents a remarkable series of adaptive changes which take place in the human body as a result of continued occupational activities. They are probably the fullest and most complete studies of this nature which we possess. One contribution, "A remarkable Example of the manner in which Pressure-Changes in the Skeleton may Reveal the Labour-History of the Individual," is a full account of the changes which appear in the skeleton of the coal-trimmer. The most notable feature is the formation of an arthrodial joint in the fibro-cartilage between the fourth and fifth lumbar vertebræ and the division of the neural arch of the fourth at two points, a result of the forcible rotation of the spine on a vertical axis which takes place when coal is thrown with great force to a considerable distance, as when the coal-trimmer is engaged at his work on board ship.

A second paper, "The Anatomy and Physiology of the Shoemaker," describes the anatomical and osteological changes which had resulted from the habitual performance of a definite series of movements entailing the expenditure of a considerable amount of muscular energy, during the greater part of a long life-time of seventy-three years. The most striking change is the formation of a buttress of bone, which extends upwards from the lateral mass of the atlas on the one side, and articulates by means of an arthrodial joint with the jugular process of the occipital bone.

Along with other papers dealing with more or less cognate subjects the two are included in a single volume under the non-suggestive title, "The Operative Treatment of Chronic Intestinal Stasis," J. Nesbet & Co., London, 3d ed., 1915.

the fundamental attributes of protoplasm. The manner of the response is adaptive, it is an individual effort, and is usually non-transmissible. Whether the responses ever become transmissible, in that they appear without the original stimulus, is the crucial point of the problem of the transmission of acquired characters. That the organism has the inherent power of forming new non-germinal characters is however not questioned, and it is well that the hard fact should be kept in mind. What we desire is some evidence that stimuli are transmissible or, if this be not forthcoming, some proof that the responses may appear without the original stimuli. At first this may be deemed to be looking for an effect without a cause, a response without a stimulus.

The callosities in the ostrich and adaptive responses generally lead one to submit that a character may become transmissible without necessarily being germinal, in the sense of having factorial representation in the germ plasm. Acquired characters are such somatic modifications as are produced as responses of the organs and tissues to stimuli, and are without direct representation in the germ plasm. In the words of Weismann: "Acquired characters are those which result from external influence upon the organism, in contrast to such as spring from the constitution of the germ."[9] They reveal an inherent power of response of the tissues and organs in a more or less definite manner according to the differentiation of the tissues and the nature of the stimulus. It may be that much of the complicated development of to-day was primarily of the nature of responses to stimuli.

The acceptance of Weismann's germ plasm theory of inheritance, strengthened as it has been by the factorial hypothesis, has for the past two or three decades concen-

9 Professor J. A. Thomson's definition (''Heredity,'' 1912, p. 173) is as follows: ''An acquired character, or a somatic modification, may be defined as a structural change in the body of a multicellular organism, involving a deviation from the normal, directly induced during the individual lifetime by a change in the environment or in function (use and disuse), and such that it transcends the limits of organic elasticity, and therefore persists after the factors inducing it have ceased to operate.'' Among his illustrations he cites: ''callosities induced on the skin by pressure.''

trated attention wholly on the germ plasm as the source of heredity and variation in the animal world. Ordinarily in studying the origin of characters we start with the germ and consider how factors arise and characters come to be formed from them; but there is no reason why we should not also contemplate their origin by observing their manner of appearance in the soma, and from this try to understand their transmissibility. Even if hitherto the former has alone proved fertile in results and the latter sterile it does not follow that renewed attacks on the problem with additional armament will always fail.

Callosities are a definite response of the skin to stimuli resulting from contact with some hard substance involving pressure and friction. They involve new inter-relationships of the structures concerned, and may affect the underlying tissues and even the bone on which they rest. On the factorial hypothesis the multitude of characteristics making up the complex organism are assumed to have a measure of independence; yet it is allowed that definite hereditary inter-relationships exist among them when we contemplate the body as a whole. May not some of the characteristics be directly factorial and others a result of the inter-relationships brought about in their establishment, just as in architecture certain subsidiary structural parts have to be introduced in order to admit of some major effect.[10] In any structural change, however simple, and whether germinal or somatic in origin, the complex tissue inter-relationships of the organism are involved. The old ties are disturbed and new ones are established. It is conceivable that a continuance of the application of fresh stimuli, from generation to generation, may result in a weakening of the old relation-

[10] Mr. L. Doncaster ("Heredity," Cambridge, 1911, p. 97) expresses much the same idea when he says: "The belief that 'somatic' changes could not be transmitted rests largely on the idea that every character is determined by a 'factor' or determinant in the germ-cell, but it is clear that any character is not developed directly from the germinal determinant, but by the relation existing between the determinant and its surroundings, viz., the body of the organism. If the surroundings are changed, this relationship may be altered, and the altered relation may be transmitted to the offspring, so bringing about a corresponding change in the character as it appears in the next generation."

ships and a strengthening of the new, until in the end one may supplant the other.

On a hypothetical conception of this kind it may be understood that the continued production of sternal and pubic callosities, generation after generation, has introduced such fixed and intimate inter-relationships of the structural parts concerned that in the end they come to replace the old inter-relationships altogether and with them the non-callous condition. The callosities are formed antecedent to and apart from the primary stimuli. Their appearance becomes accelerated, as it were, and they arise even before the chick is hatched and the original stimuli can be effective. They are not new characters which have come in, but are new as regards the ontogenetic time at which they appear.

The possibility of responses occurring without the original normal stimuli may be illustrated from certain of the instinctive sexual activities of the ostrich. At the breeding season the cock bird performs the sexual display known as "rolling." He crouches on the ground and with wings outspread rolls from side to side, his long neck and head also taking part, the latter striking vigorously against each side of the body alternately. Also as he approaches sexual ripeness he begins to "bromm," the sound having often been compared with the roar of a lion. The mouth being closed he inflates the esophagus until the neck as a whole becomes two or three times its usual thickness and then forcibly expels the air through the nasal passages, producing a booming noise of great carrying power, consisting of two short notes and a long one, the sequence being repeated from one to six or seven times, and serving as a guide to the farmer as to the state of sexual ripeness of the bird. Again, during actual pairing, the cock mounts upon the back of the crouching hen with his right foot upon her back and the left upon the ground, and sways the front part of the body and neck to and fro as the act is consummated.

The above are three distinctive actions on the part of the cock ostrich which are usually performed only at sexual maturity, and may be deemed to be responses asso-

ciated with stimuli from secretions or enzymes of the sexual organs. Yet occasionaly very young chicks, perhaps only a week or two old, are to be seen performing the same, though in an imperfect manner. They can "roll" almost perfectly; a chick can inflate its neck, but has insufficient strength to expel the air with enough force to produce a "bromm"; and often one chick will attempt to mount another which is resting on the ground, and begin to sway from side to side in a ridiculous fashion. May not these precocious activities be interpreted as an acceleration of responses normally due to stimuli of a sexual nature? Now they are performed wholly apart from the usual stimuli and are of no adaptive nor selection value at this early stage. They have become, as it were, so integral a part of the organism that they break out without the original stimulus; they have become transmissible. They are hardly sufficiently general to be comprised under the term "play" and, in the sense of Carl Groos, to be regarded as preparatory to the real business of life. Probably many activities of a similar precocious nature could be brought forward where an intensive study of an animal has been made. They serve to show that a physiological action is not necessarily a response to the stimuli which originally called it forth; but may appear antecedent to and independently of them.

Just as physiological activities may make a precocious or accelerated appearance so it may be that acquired, morphological characters at times appear in advance and apart from the stimuli which originally called them forth; they may become transmissible, though not germinal in the factorial sense. It is submitted that the formation of callosities, ordinarily developed as responses to pressure and friction in the life-time of the individual bird, has become thus accelerated, so that they arise at a much earlier period, even within the egg, and apart from the usual stimuli. Arising in this way a character is not germinal in the sense of having factorial representation, but is nevertheless transmissible. Though appearing before hatching it is no more germinal than it would be if developed as a definite response to the post-natal stimuli

of friction and pressure. On this interpretation a new character, to wit, a callosity, can arise either before or after hatching as a result of the responsive nature of the tissues, apart from any germinal representation.

Acquired adaptive characters, structural responses to internal or external stimuli, are by their very nature extra-germinal, and their appearance may well lead us to hesitate in accepting the germ plasm theory as a complete interpretation of everything somatic, or of everything that is transmitted from generation to generation, despite the statement by Dr. C. B. Davenport[11] that: "Upon one point all geneticists are, however, agreed . . . that we must interpret all our results in terms of genes alone."

So plastic and so responsive are the parts of the organism to stimuli that, in spite of such an embracive pronouncement, it may still constitute a subject for enquiry whether many of the adaptive relationships in organisms are not such as were originally impressed upon the individual as a result of its activities or subjection to former stimuli and which have in time become transmissible. The problem has been neglected for the past two or three decades as a result of the firm hold which the germ plasm theory of inheritance has gained over the minds of biologists and the general acceptance of the non-heritability of acquired characters. *Renewed search will probably disclose many other instances of characters appearing pre-natally which could just as well be developed as needed in the life-time of the individual, and thereby throw suspicion upon their germinal origin.* Callosities are undoubtedly the most direct and simple instances of this nature which could be adduced; we have both transmissible and non-transmissible examples in the same individual. Those whose transmissibility is established could have been formed post-natally just as readily as those produced where pressure and friction are applied to surfaces not already callous. Knowing also the responsive nature of muscles, tendons, ligaments and osteological tuberosities and the readiness with which they are modified through change of habits, it is not improbable

that many now regarded as transmissible could also arise as needed as direct responses. It will certainly be legitimate to question the germinal origin of those characters whose formation can be interpreted as adaptive responses to changes to which the organism is subject.

The germ plasm theory of Weismann and the factorial hypothesis of Mendel, Bateson and others have been of inestimable value in enabling us to appreciate many of the facts of heredity. But no one imagines that they give us the completed account of evolution and adaptation, as many are beginning to feel now that their contributions can be estimated more or less in their entirety, and we get a true perspective of what they have to offer. They are and will remain important chapters in the story of variation, heredity and evolution, but they are not the whole volume; nor are they the concluding chapters, as their supporters themselves would doubtless admit. It is submitted that something is yet to be gained from consideration of how adaptive characters arise as a result of stimuli from use and disuse of parts and from environment, and how they may become transmissible, though not necessarily germinal. The germ plasm theory to a large extent and the factorial hypothesis *in toto* are sterile when we come to questions of adaptation, and natural selection has to be freely invoked, whereas practically every structure in the body bears witness to its adaptive nature.

For an acquired character to become transmissible, so that it appears independently of the stimuli which originally called it forth, is manifestly a difficult proceeding when regarded from the point of view of the hereditary structural relationships which have been established through long ages. The natural and experimental phenomena of regeneration show how deep is the tendency to maintain the established relationships of the various parts of the body. An acquired character represents some temporary disturbance of the normal relationships, but ordinarily the old correlations return with the next generation and the new are but transient, persisting for the generation only. When however these

new relationships are repeated generation after generation and maintained at their full vigor for the whole lifetime, it is conceivable that they become so impressed on the organism that they gradually overcome the old weakening relationships of parts and appear from the beginning in place of them, in other words, the character becomes transmissible, the new ties become the heritage of the organism. This, of course, is no proof of the inheritance of acquired characters, but may help us to conceive its possibility in the light of considerations engendered by the callosities in the ostrich.

The skin is more likely to show responses to environmental stimuli and to the general activities of an animal than the internal organs on account of its superficial, exposed position, and callous pads are among the simplest of structural responses and their formation is readily understood. Where temporary, as on the human hand, they are by no means likely to impress themselves permanently as new interrelationships on the surrounding parts. Where, however, as in the ostrich, they would form from the beginning and persist throughout life, from generation to generation, it is more conceivable that they would impress themselves on the constitution of the bird and their time of appearance would undergo acceleration with an independence of the primary stimulus.

The accessory, non-transmissible callosity at the ankle has not yet impressed itself so forcibly upon the general structural relationships as permanently to disturb the normal tendencies, and it has to be formed anew in each generation from direct stimuli. The hereditary median thickening is the primary one, and may well justify us in thinking that the three-toed, ancestral stage of the ostrich was of long geological duration; the new pad formed by the two-toed bird is more recent and has failed as yet to attain transmissibility. It may be that in its early days a race is more responsive to adaptive, structural changes than at a later period. In many respects the ostrich now appears senescent, and may well be expected to be less plastic than in past ages.

In general, correlated structural relationships, established through long ages, will act as a *vis inertiæ* to the introduction of acquired changes; they will represent so much heritable, inherent tendency which has to be overcome before any new relationship of parts can be established. Life-time changes of habit or of environment, as in the assumption by man of the erect habit, or the taking to water of a former terrestrial organism, are the conditions which will be conducive to acquired changes becoming transmissible, compared with those under which the responses are temporary, or continued for a few generations, or are the result of mutilation.[12]   Any temporary structural relationship established, as in the decaudation experiments of Weismann and others, would manifestly be incapable of overcoming those deeper relationships which, with each new generation, find their expression in a complete tail. As Professor T. H. Morgan[13] points out, the theory of the inheritance of acquired characters "is one that has the great merit of being capable of experimental test," but he allows that "modern Lamarckians are justified in claiming that the validity of the theory can only be tested by experiments in which the organism is subjected to influences extending over a considerable period." The hypothesis here submitted is undoubtedly one which in most experimental cases would demand long period for the effectiveness of its tests.

We need not expect mutilations to become transmissible, nor most of the responses established during the life-time of an individual; but this in no way precludes the possibility for life-time responses which are continued for generations, or which may happen to strike a race at some plastic period of its existence.

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[12] In the adoption of a new habit during the life-time an adaptive character may appear from generation to generation as the habit comes to be assumed, and give the appearance of being transmissible, whereas it may be formed as an ordinary response to the new stimuli. Especially where an animal is in process of changing the stimuli to which it is subject will it often be difficult to distinguish a transmissible from a responsive adaptive character which is non-transmissible.

[13] Morgan, T. H., "Evolution and Adaption," 1903, p. 230.

It is by no means anticipated that the conception of the transmissibility of characters as so many accelerated adaptive responses, involving new structural inter-relationships, and not necessarily with factorial germinal representation, will apply to all the features of an organism and serve as an explanation of the origin of heritable characters generally. Its application may be limited to such as have an adaptive significance, and can be assumed to have arisen in the first instance as a result of internal or external stimuli acting upon the soma. As will be shown in a later paper the ostrich itself, especially in the details of its degeneration, presents us with many character changes which have manifestly no adaptive significance, but are the expression of germinal changes, uninfluenced by external forces. Without question we are short-sighted in attempting to reduce the methods of evolution to some common term; as Professor H. F. Osborn points out in his new book: ''The Origin and Evolution of Life,''[14] there are *centripetal* factors in organic evolution, there are *centrifugal* factors. Much of the recent work on Mendelism and mutation strongly supports the view so warmly advocated by Professor W. Bateson and Professor T. H. Morgan that germinal characters appear apart from any adaptive considerations, and the degenerative changes in the ostrich are in full accord with this; but it is by no means a complete answer to the problems of evolution, where so much appears that is directly adaptive and so little that is non-adaptive. Most genetical work during the present century has been unconnected with adaptation, yet it is one of the big problems of biology which calls for solution as insistently as ever, and it may be that a proper interpretation of the callosities in the ostrich will assist in some measure towards an understanding.

[14] Reviewed by Professor Lillie in *Science*, November 8, 1918.

THE SELECTION OF FOOD-PLANTS BY INSECTS, WITH SPECIAL REFERENCE TO LEPI-DOPTEROUS LARVÆ[1]

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THE instinctive behavior exhibited by phytophagous insects in the selection of their food-plants is always a matter of interest to entomologists, and it is one of the fundamental principles underlying the application of entomology to agriculture, horticulture and forestry. Nearly all insects show a great fixity of instinct in this respect, but a most cursory examination of the habits of almost any group will reveal a considerable variation among different species, particularly with reference to the number of plants regularly utilized as food and in the selection of closely related or of very diverse plants.

The origin and development of the association between insect species and plant host has been the basis for a considerable amount of speculation which has increased in proportion to the additional knowledge continually added through field observation, collection, and rearing of insects.

Before considering any of the theories advanced to account for the association of insects with definite plants, I shall attempt to give a very brief account of the salient facts concerning food-plants which appear to be sufficiently definite for orderly arrangement, restricting the discussion for the present, mainly to one of the better known orders of insects.

The term phytophagous with reference to insects is commonly employed in a considerably restricted and rather inaccurate sense, including only those species that feed upon the higher plants, meaning by these the ferns

and flowering plants. Only an extremely small, almost negligible, proportion subsist upon ferns, so that from a practical standpoint, we would include only those feeding upon the Spermatophytes. This usage has developed on account of the fact that the fungi which have many insects feeding upon them, do not ordinarily engage the attention of the economic entomologist, and for convenience it is acceptable in the present connection, as very little is known concerning the specific hosts of insects living in fungi. Furthermore, the food-plant is ordinarily understood to mean the species upon which the larval or growing stages occur, for although it is common to find both the young and adult insects of the same species subsisting upon the same plant, it occurs also very frequently that the food of the larvæ and imagines of holometabolous insects is of entirely different nature. Among the many other truly phytophagous insects living in fungi are a number of families of beetles, for example, which develop in the tissues of the larger, fleshy fungi and many of these mycetophagous insects undoubtedly show a very close association with certain species of fungi. In addition, some insects subsist upon the lower fungi, yeasts and even bacteria. The biology of these latter is very imperfectly known in nearly all cases, owing to the greater difficulties attendant upon studies dealing with them. The well-known fungous-growing ants and termites and the ambrosia beetles actually cultivate certain fungi for food and other insects (undoubtedly a far larger number than is now known) subsist upon various microorganisms, although they are, to the eyes of the casual observer, feeding directly upon the substrata which really nourish the microscopic fungi, yeasts or bacteria, that in turn form the actual food for the insects. As already said, however, these symbiotic relations are in most cases only very poorly understood, and they are entirely outside the scope of the present discussion.

As distinguished from those of predatory, parasitic and saprophagous habits, the phytophagous insects rep-

resent probably nearly half of the known species, and a
considerable proportion of the several orders of insects
contain at least some species that are phytophagous in
the sense indicated above. Some of these, like the Or-
thoptera, are very primitive, while others of probably
equal or even greater antiquity are not phytophagous, so
that it is difficult to say whether the earliest true insects
were vegetarian, predatory or saprophagous.[2]   This
question is perhaps not a very important one, for, as will
be pointed out later, a change from one type of food
habits to another has actually taken place independently
in several families of the highly specialized Lepidoptera.

As we might naturally expect, it is possible to point
out in a very general way a progressive specialization
in the selection of food-plants which parallels to some
degree what appears to have been the path of evolution
among insects, as determined from the criteria fur-
nished by comparative anatomy, development and pale-
ontology. Thus, the primitive Orthoptera appear to se-
lect their food-plants with but little discrimination, while
the Lepidoptera and phytophagous Hymenoptera exhibit
almost unerring accuracy in their instincts to choose cer-
tain plants and consistently to ignore all others. Beyond
this, however, it is not easy to make any broad state-
ments, for among the most highly specialized groups we
find a great variability, at least in the number of food-
plants admitted to the menu, as well as in regard to the
botanical relationships of the plants regularly selected.

It may be argued that selection of food-plants is a
somewhat dubious expression and that it may not accu-
rately represent the condition of affairs from the stand-
point of the larval insect. In most cases the larval food-
plant is really chosen by the adult female, who places her
eggs upon certain plants which then become of necessity
the food of the resulting larvæ, which could not very
readily migrate to another kind of plant even should they

[2] This last term is rather ambiguous and is rapidly becoming still more
so in the light of studies recently made upon insects that subsist upon
microorganisms.

be willing to do so. This objection is readily met by experimental evidence, for every entomologist is fully aware of the fact that it is ordinarily quite impossible to rear insects of restricted food habits upon other than their normal food-plants. It is true that an acceptable plant may sometimes be found by those familiar with the vagaries of related species of insects, but in such cases we may safely assume that the experimentally selected plant may later prove, in at least some cases, to be one sometimes picked out for food in nature.[3] It would be an unwarranted assumption, therefore, to suppose that the maternal instinct of oviposition does not at the present time represent fairly well the tastes of the larva. We may reasonably ask, however, whether the selection of the mother may not have impressed itself upon the larva after continual repetition or whether the taste acquired by the continual feeding of the larva may not persist into the adult, just as fondness for sweets may become a lifelong attribute in examples of the human species pampered in youth by indulgent mothers. During the progress of evolution as food-habits have become fixed, it is evident that any changing tastes on the part of the larva must have become a part of the egg-laying instincts of the mother, through the action of natural selection or otherwise, before any change of food-plants could occur. On the other hand, any change in the instincts of oviposition, not incompatible with larval tastes, might quickly become a definite characteristic of the species. If any adults should select unsuitable plants their progeny would quickly perish. The maintenance of definite preferences can thus be seen to be readily perpetuated through the action of natural selection in the survival of the fittest strains and the elimination of the unfit ones. It will be evident later, however, that subsistence on many food-plants would appear to have originated after

[3] It may also be noted that those experienced in rearing caterpillars are frequently able to rear species of unknown habits on certain plants (*e. g.*, chick-weed, *Cerastium*) on which they do not normally feed, but which are acceptable to many larvæ in the absence of their natural food-plant.

the manner of mutations, and it will, I think, be evident
that we should attribute these, at least in part, to chance
mutations or aberrations of instinct in the parent insects.

Before dealing specifically with the selection of food-
plants, it is necessary to classify in a general way the
types of food-habits generally met with in insects. Thus,
Reuter applies the terms Pantophaga to omnivorous in-
sects, Phytophaga and Sarcophaga to vegetarian and
carnivorous ones respectively and Necrophaga and Cop-
rophaga to those living upon dead animals and excremen-
titious material. Among the Phytophagous forms he
would further distinguish monophagous and polyphagous
species on the basis of the number of food-plants which
they utilize. Although satisfactory so far as it goes, this
fails to include several categories commonly referred to
by entomologists and for the present purpose it can be
readily enlarged as follows:

	Pantophaga	
Phytophaga	Sacrophaga
Monophaga	Harpactophaga
Oligophaga	Entomophaga
Polyphaga	

Saprophaga

(Partly subdivided as below)

{ Microphaga }	{ Necrophaga }
{ Mycetophaga }	{ Coprophaga }

In this arrangement a distinction is made between
vegetarian species with a single food-plant (Monopha-
gous), those with several definitely fixed ones (Oligo-
phagous) and those with quite indiscriminate food-habits
(Polyphagous). On the other hand predatory species
(Harpactophagous) and entomophagous parasites are
distinguished, as each form a very large and important
group. Many necrophagous and coprophagous species
really subsist on bacteria, fungi, etc., and these may
perhaps be better designated as microphagous and
mycetophagous.

Among phytophagous insects, the polyphagous habit is
probably the most primitive and the monophagous one

the most highly specialized. It is not rare, however, to find all three types represented in otherwise very homogeneous groups. The Lepidoptera, for example, form an enormous complex of species, practically all of them phytophagous, the majority feeding upon a very restricted series of plants and representing the oligophagous habit, with a smaller series of apparently monophagous forms and a few secondarily polyphagous ones. Fortunately also, the food habits of this order as a whole, are better known than those of other insects and it can be examined with less chance of error than perhaps any other group of equal extent.

As already stated, nearly all of the larvæ of the Lepidoptera are phytophagous at the present time and there can be no question that since the order has existed this condition has prevailed. Owing to a change in the form of the trophi during metamorphosis by which the adult Lepidoptera develop haustellate or sucking mouthparts, the food of the imagines is entirely different from that of the larvæ and they subsist upon liquids, mainly the nectar of flowers.

We may then classify the food-habits of the larvæ roughly as follows:

Food Material	Frequency of Utilization
Plant-food	Nearly all of the species
Bacteria	Probably none
Fungi	Almost none
Lichens	A very few, mainly in one family
Mosses	Almost none
Ferns	A very few
Flowering plants	Probably about 99 per cent.
On foliage	A large majority
In flowers	A few
In aquatic plants	A very few
In roots	A few
In tissue of herbaceous plants.	A rather small number
In wood of shrubs and trees...	A rather small number
In dried seeds, fruits, etc.	A very small number mainly in one group
Animal food	
On other living insects	A few isolated cases
On material of animal origin;	
wool, horn, beeswax, etc.	A very few, mainly in one group

From this it will be seen that while the food-habits are very homogeneous, isolated cases occur where certain species have departed very strikingly from their more conservative relatives. Among these, the most interesting are those which have become carnivorous. Thus, we have in the eastern United States, a small butterfly, *Feniseca tarquinius,* which feeds upon plant lice occurring on alder. In our southwestern states there occurs also a moth of the genus *Epipyrops,* typical of the family Epipyropidæ, utterly unrelated to *Feniseca,* which feeds upon Homopterous insects of the family Fulgoridæ, and other species of *Epipyrops* are known to have quite similar habits in the orient. Also *Thalpochares,* a moth of the family Noctuidæ, is known to feed upon aphids and scale insects in Europe and Australia. Similarly the caterpillars of the Australian Cyclotorna is ectoparasitic on Homoptera of the family Jassidæ, and the larvæ of *Zaphiodiopsis* feed upon other caterpillars. A still further and more extraordinary modification is in the larva of the British butterfly, *Lycæna arion,* which is herbivorous in its early stages, but enters the nests of ants to prey upon the ant-larvæ during its final period of growth. Other scattered cases of predatory caterpillars are known, including other butterflies and moths of several families. With these the most striking feature is that the prey almost always consists of Coccids or Aphids. This association is probably due to the fact that these Homoptera are sessile or slowly moving creatures, commonly present where caterpillars occur and therefore apt to attract those of carnivorous instincts. Of interest in cennection with this, is the fact that certain phytophagous caterpillars may become temporarily carnivorous, quite regularly or under the stress of circumstances. Thus, the very abundant and destructive corn ear-worm, *Heliothis obsoleta,* commonly lays a number of eggs on the silks of a corn-ear, although nearly aways only one caterpillar finally survives in the interior of the ear where it does most of its feeding. Here the elimination

is due to a cannibalistic instinct of the caterpillar which results in the disappearance of the excess individuals, notwithstanding the fact that there is food enough for a considerable series in a single corn ear. A similar cannibalistic habit has been reported in *Hadena* and *Agrotis,* two other genera of the same family, and no less than 75 species of European Lepidopterous caterpillars are known to be occasionally predatory through temporary aberrations of their trophic instincts.

With such plasticity of behavior in several diverse families and even with *Lycæna arion* and certain small moths exhibiting a change in food habits during ontogeny, it is not difficult to regard the origin of sarcophagy in Lepidoptera as due to independent changes which have become firmly fixed in individual species or genera.

The habit of certain Tineid moths, including the clothes-moth (*Tinea*) and some of its relatives, to feed upon wool and other materials of animal origin is well known, and other non-domesticated forms of the same group exhibit similar food-habits. One African species of *Tinea* lives at the base of the horns of a large water antelope, where it forms tubes similar to those constructed by some other Microlepidoptera. The bee-moth, *Galleria mellonella,* a commensal in the hives of the honey-bee, subsists upon beeswax and bits of refuse said to contain about 20 per cent. of nitrogenous matter. Practically all of the caterpillars that subsist on foods of animal origin are more or less closely related, but not sufficiently so for us to entertain for a moment the belief that the habit has not originated independently in numerous instances. Why it should be restricted to a few groups in one part of the order, may, I think, be explained on the following basis. Among the Microlepidoptera only do we find forms able to subsist upon plant materials containing a very small amount of water (*e.g.,* seeds, dry fruits, grain, flour, etc.) as distinguished from the tissues of growing plants. Even in the wood of trees,

tunneling larvæ remain in a moist burrow where evaporation is very slow. Similarly the animal materials utilized as food are very low in water content. That we do not find Lepidopterous larvæ in moist material of animal origin is no doubt due to the fact that they do not appear to be adapted for subsistence upon the abundant micro-organisms present in such materials.

Passing to a consideration of the phytophagous Lepidoptera, by far the greater part of the order remains to be dealt with. As indicated in the tabulation, practically all of these occur on the higher plants and feed almost always upon living tissue. The latter is true almost without exception of the leaf-feeding forms, although one of our common moths of the eastern states, *Pyromorpha,* is known to live upon dead and decaying fallen leaves and another of our small moths avails itself of hemlock chips. Among those which live in woody tissue, some prefer weakened or sickly trees or unhealthy branches, but almost none occur in dead wood.

Of those living on the lower plants, one small family of moths, the Lithosiidæ, subsist upon lichens and they are almost the only ones affecting these plants. This family is far from primitive, so that its association with a series of lower plants could have no significance, even if it were definitely known that the lichens are a very old group, which does not seem probable.

Mycetophagous forms of Lepidopterous caterpillars are of very unusual occurrence, in spite of the fact that several large series of beetle larvæ develop in fungi. They are found, however, and there are in North America at least two species of *Tinea* which have been bred from these plants.

In spite of the similarity of their foliage to that of the flowering plants, ferns do not commonly serve as food plants for insects. They are, in fact, strikingly immune from insect pests of all sorts. This is hardly what might be expected from the long presence of this group of plants, their enormous development in the past, and their

persistence at the present time in quite considerable abundance. Why they should be so sparingly selected as food plants does not seem to have been adequately explained.

The use of Phanerogams as food-plants is so general that it is possible to gain a much clearer insight into the conditions pertaining to them than is the case with other plants. In general the food habits of butterfly larvæ are more fully known than those of the moths, on account of the smaller number of species and the general interest taken by amateurs in this group.

An account, very complete at the time, has been given by Scudder of the food-plants of the butterflies of eastern North America.[4] A tabulation of the food-plants included in this list shows several interesting features. Fifty-five families of plants are included (not taking into account several larvæ feeding on conifers and our one predatory species) and the list contains a very representative series, drawn from both the Monocotyledons and Dicotyledons in approximate proportion to the number of species of these two sections. It is noticeable, however, that several common families, the Iridaceæ, Orchidaceæ, Caryophyllaceæ, Euphorbiaceæ, Vitaceæ, Primulaceæ and Rubiaceæ are entirely omitted, that only one species occurs in the Labiatæ, or on the Umbelliferæ, and that only a very few affect Compositæ. We may readily see that the generally strong-scented Labiatæ and Umbelliferæ and the milky Euphorbiaceæ might require great adaptation on the part of larvæ eating them,[5] but the omission of the other families if not entirely a matter of chance must rest upon some less evident basis than the foregoing. Among the other plant families the number of species of caterpillars compared to the number of eastern American genera, included in each family that is

[4] This list has been used as a whole as it is complete in itself, and to attempt to add to it and emend it by the present writer, from more recent literature, would improve it but little for the present purpose.

[5] This is true only of the butterflies in this list; many other leaf-eating larvæ feed in abundance on these plants.

fed upon, varies exceedingly, from 1:100 to 1:1 or even 1.6:1 in the case of the Rutaceæ. The average is about 1:4, but there is no tendency for the ratios to fall near the mean and their distribution if not a matter of chance, must have been determined in relation to their environment, no doubt to a great extent by their struggle for existence with other plant-eating forms.

If we examine the food-plants of the genera or higher groups of butterflies, we find that most of them exhibit well-marked preference for certain, usually related plants. The food-plants of the British butterflies are unusually well known, and Tutt has recently given in his work on "British Butterflies" a digest of their preferences which he finds to be closely similar to those of the nearctic forms previously considered by Scudder. Gathering his more definite data together and using his terminology for the groups, the food-habits may be tabulated as follows:

Vanessids ....Urticaceæ Compositæ, etc.
Argynnids ....Violaceæ exclusively
Brenthids ....Violaceæ generally
Melitæids ....Various plants
Pierids ....Cruciferæ essentially
Anthocarids ....Cruciferæ essentially
Limenitids ....Various plants
Apaturids ....Various plants
Satyrids ....Gramineæ almost entirely
Coliads ....Leguminosæ almost entirely
Gonepterygids ....Rhamnaceæ
Ruralids ....Various plants (more or less fixed in
                                         some subdivisions)
Lycænids ....Diverse food-habits
Chrysophanids ....Polygonaceæ almost exclusively
Urbicolids ....Various plants
    Sub-groups Thymelicines ....Gramineæ mainly
              Urbicolines ....Gramineæ mainly
              Cyclopidines ....Gramineæ mainly
              Hesperiines ....Leguminosæ and various other plants
Papilionids ....Various plants; several groups with
                                   particular food-plants

Viewed in this way, it is seen that barring many exceptions, there is a general tendency, much more pronounced

in some groups than in others, to select plants of very
specific families or even genera. This must not be under-
stood to mean that the individual species of insects affect
indiscriminately many or all members of the plant group,
but that their normal food-plant or plants do not fall out-
side the group. With the exceptions in mind, the fixity
of the instinct to feed on only certain kinds of plants is
all the more extraordinary, for we cannot readily dismiss
it as a physiological or nutritional necessity.

An interesting light upon the effect of the environment
in influencing the selection of food-plants, is furnished
by several widely distributed species and genera of but-
terflies. Thus several species of *Vanessa* have quite
identical food-habits throughout the entire holarctic re-
gion and the same is true of several very closely allied
palæarctic and nearctic species belonging to the same
group, while, as mentioned above, the general food habits
of the larger groups run closely parallel among their rep-
resentatives on the two continents. Still more interesting
in this respect are the butterflies of the closely related
genera *Catopsilia* and *Callidryas* which restrict them-
selves to the Leguminous genus *Cassia*. These butter-
flies occur in the nearctic, neotropical, Indo-Malayan and
Australian regions and such species as have been reared
show this preference, which is probably universal. The
well-known genus *Papilio* supplies some similar peculi-
arities in that several world-wide groups of the genus
are restricted to certain closely related groups of plants
(*e. g., Aristolochia, Citrus,* etc.). On the other hand, one
North American species, the common *Papilio glaucus,* is
known to affect food-plants belonging to no less than fif-
teen different families of plants. With such constancy
in the most remote quarters of the globe among related
species of this genus and with one species in a single
region regularly developing on the most diversal plants,
we must believe that the fixed instincts of some species
are not to be led astray by the many temptations offered
even by the varied plants of widely separated zoological

regions, while those of other species are so loose that
they restrict their owners only to a comparatively very
small extent. Such conditions certainly point to instinct
as the determining cause of food selection, rather than
physiological adaptation to specific kinds of plants.

In connection with cosmopolitan butterflies, Scudder
noted many years ago, that there are no species of re-
markable distribution known to feed upon Leguminosæ
or grasses, although these plants are cosmopolitan and
harbor many species. I am inclined to believe, however,
that this has no significance, particularly in view of the
aforementioned Coliads that feed upon *Cassia* in various
parts of the world.

Many other groups of Lepidoptera conform quite
closely in food-habits to the butterflies, although some
show greater diversity, especially in affecting different
parts of the plant, and it may be said in general that the
larger moths are less apt to be monophagous than the
butterflies.

Among the hawk-moths or sphinx-caterpillars several
subfamilies show a restriction to groups of related plants,
while others do not. Thus in this cosmopolitan family,
one subfamily (Chærocampinæ) feeds on Vitaceæ, with
an admixture of diverse other plants, another (Macro-
glossinæ) on Caprifoliaceæ exclusively, another (Sphin-
gineæ) to a great extent on Oleaceæ together with other
plants as different as Conifers, Solanaceæ, Euphorbiaceæ
and Labiatæ, while one (Smerinthinæ) exhibits no appar-
ent preference.

The family, Aegeriidæ, or clear-winged moths, live in
the larval stage in the interior of plants, tunneling
through the tissue. They affect a very wide series of
plants, herbs, shrubs and trees, as can be seen from
the following abbreviated list which represents the range
in habits of some of our eastern North American repre-
sentatives; stems of Cucurbits, *Vernonia,* blackberries,
currant, grape; wood of pine, willow, lilac, maple, oak,
peach; roots of *Clematis,* persimmon, blackberry, *Eupa-*

torium; stumps of oaks cut the previous year; and oak-galls. Notwithstanding such diversity within the family, the individual species are nearly monophagous, or oligophagous on related plants. Such a condition would seem to have arisen through sudden mutations in instinct rather than from numerous smaller variations having a selective value, for in the latter case we should find polyphagous forms developing in some places at least.

Another, much smaller family of rather generalized structure, the wood-boring Cossidæ, have habits similar to many of the Sesiidæ, but their selection of food-plants is very different. Typically they are oligophagous, but some species, including the well-known and destructive shade-tree pest known as the leopard-moth (*Zeuzera pyrina*) introduced from Europe into the eastern United States, has been bred in this country from an almost endless variety of shrubs and trees, as it has been in Europe also. As listed by Chapman, the American food-plants belong to twenty-two families of plants and to nearly fifty genera. Almost all that can be said of the leopard-moth's bill-of-fare is that it includes no conifers.

Cossus ligniperda, another European species is strongly polyphagous, but many of the exotic species appear to avail themselves of a rather restricted diet. As this latter is perhaps due to lack of knowledge, it may be unwise to draw any conclusions at present.

In other families of moths the same phenomenon is frequently encountered. The large group of Noctuidæ, comprising the owlet-moths, feed mainly upon the foliage of a wide rang of plants, while the list of food-plants for the numerous species varies greatly in extent. To attempt to classify the food-habits of this group would require much time and space, but it may be said that there are species in certain genera, as, for example, the cotton boll-worm, which appear to have rather suddenly enlarged their range of food-plants as compared with that of related species of the same genera.

In regard to the uniformity of food-plants during on-

togeny, the statement has been made that some Lepidop-
terous caterpillars occur on a greater range of plants
when young, or at least that they will readily feed upon
certain kinds during the earlier instars, and refuse them
later, so that their diet becomes more restricted as
growth progresses. This statement has been in turn used
as an argument that oligophagous forms are derived
from more restricted feeders, and that they repeat in a
way their history by the limitation of their food-plants
during successive instars.

Some elaborate experiments on the feeding habits of
the gipsy-moth reported on by Mosher tend to discredit
this supposition, however. As is well known, the gipsy-
moth occurs on a wide range of plants, but shows well-
marked preferences for certain among them which repre-
sent its favored food. These experiments were carried
out in the extensive detail possible only when dealing
with insects of great economic importance, and although
planned for another purpose, furnish valuable data upon
this point. It appears that on a number of their numer-
ous food-plants, the gipsy-moth caterpillars show an in-
ability or at least an unwillingness to feed either during
the very early or during the later larval stages. On
some kinds of trees the early larvæ failed to develop and
on others the latter stages did not feed, although the
young ones did so. This diversity of behavior is in part
due to the fact that young larvæ cannot usually feed upon
conifers, while the older ones eat the foliage of these trees
voraciously; but it is by no means due to this alone, so
that we can say that the juvenile preferences of the larvæ
become transformed or changed as growth progressed.
With an active polyphagous caterpillar like the gipsy-
moth in which the larvæ often migrate to other species of
plants during growth, it is possible for such changes in
diet to take place regularly in nature, although such could
not ordinarily occur with oligophagous species without
tending greatly to reduce the chances of the species to
survive. As the necessity for migration is most acute in
the case of very abundant species, they are open to more

temptations to avail themselves of a variety of foods and we find that it is usually the most abundant species of any group that are polyphagous. Conversely we may say also that polyphagy, when present, greatly increases the chances for the larvæ to secure the necessary amount of food for complete growth and tends to cause the species to become excessively abundant. Once under headway, these two processes will act together and result in the production of dominant species that tower above their fellows. Examples of this are seen in the gipsy-moth,[6] the *Cecropia*-moth, the army-worm, *Papilio glaucus* mentioned above, the woolly bear (*Isia isabella*), etc. We must not lose sight of the fact, however, that this is only one of many factors influencing dominance. The milkweed-butterfly, one of our most abundant native species develops on a very common plant (*Asclepias* almost exclusively) and is thought also to be a protected species.[7] Its dominance may be interpreted, like that of many destructive agricultural pests, as due to a plentiful and unfailing food supply, coupled with other pre-requisites inherent in the insect itself.

In spite of the many exceptions and variations which have been enumerated, the fact stands out clearly that the Lepidopterous insects show a very fixed instinct to select definite plants for larval food; that many are extremely precise in this respect, some less so, and others quite catholic in their tastes. Furthermore there is much to show the existence of a so-called "botanical instinct" in species, genera and even families, whereby evidently related plants and these only serve as food. A few species have departed from the general habit so far that they have become carnivorous, and among the others we find every gradation between the extremes of monophagy and polyphagy.

It has been claimed that the food habits may be modified experimentally, in that caterpillars reared on a

[6] This is true also in the native habitat of this species, aside from the decimation by parasites which occurs more abundantly in Europe.

[7] *i. e.*, distasteful to its enemies and exhibiting warning coloration.

strange plant (where they could be induced to select it)
give rise to moths whose progeny more readily accept
the new plant. It is very difficult to accept such evi-
dence, at least as having any general application, with-
out very clear and incontrovertible proof. If such trans-
formations can occur so easily and become hereditary so
quickly they should have entirely destroyed the coherent
habits now existent, during the enormous period which
has elapsed, for example, since the violent-feeding Ar-
gynnids were differentiated, since the holarctic and nearc-
tic Vanessids have been separated, or while the world-
wide Aristolochia-feeding Papilios were attaining their
present distribution. That such a change has actually
occurred in the case of other groups seems equally evi-
dent, although, as has been shown, we can more easily
believe that they may have arisen through mutations in
maternal instinct not incompatible with larval tastes and
then only in extremely rare cases and confined to certain
groups.

With a knowledge of the specificity of proteins in dif-
ferent living organisms and their apparent differentia-
tion as a replica of the genealogical history of the animal
and plant kingdoms, has come the suggestion that the
dependence of monophagous or oligophagous insects
upon specific plants rests upon a physiological basis, and
that particular proteins or vitamines are an actual neces-
sity for growth and development. A survey of the field
does not seem to bear out this supposition, however
plausible it may appear at first sight. With monopha-
gous larvæ, it will serve as a reasonable explanation, and
with oligophagous ones also so far as the individual spe-
cies are concerned, especially where such species select a
series of related plants. With those that select only a
few plants, however, and at the same time such as are
evidently not closely related, it does not seem so appro-
priate. It is when we compare the lists of food-plants of
several oligophagous species that it appears to fail com-
pletely to meet the requirements. Thus we find, referring
again to our North American butterflies, such combina-

tions as Leguminosæ with Pinaceæ, or with Rhamnaceæ, Polygonaceæ, Cupuliferæ in the case of different larvæ. More uniformity should certainly be expected if the selection of diverse plants depended upon the actual chemical characteristics of the plant tissue. We should have also to assume that the digestive functions of the cecropia caterpillar with its sixty-odd food-plants were fundamentally different from those of monophagous caterpillars.

There is much in the behavior of certain species to suggest that food-plants are selected on the basis of odor by the parent female and also accepted on the same basis by the larvæ. Experiments with cabbage butterflies by Verschaffelt and others show that these insects are attracted by the mustard oils present in these plants, and it has also been shown that caterpillars will feed on other plants which have been treated with one of these oils. Similar behavior in the most diverse insects is also known in the attraction exercised by specific fermentation products (*e.g.*, to *Stomoxys, Drosphila,* etc.). The distaste of mosquitoes for oil of *Citronella* is well known, as is also the attractiveness of this same substance for fruit-flies of the genus *Dacus.* That the same cabbage butterflies have definite dislikes in the way of plant odors has recently been claimed by the Russian entomologist Schreiber, who found that *Pieris brassicæ* would not attack cabbages planted in close proximity to tomatoes. *Pieris rapæ* does not seem to behave similarly, however, and this dislike is probably not general among the crucifer-eating Pierids.

Very recently McIndoo has published some observations showing that caterpillars readily react to the odors of several essential oils and to those of various plants. This, taken together with the fact that *Pieris* will feed upon strange plants treated with mustard oil, would suggest that odor is an important factor in the selection of food-plants. Queerly enough, however, he found that the response to their own food-plants was no more rapid than to the other substances, and even slower in some

cases. As the smearing of the oils of one plant on an-
other does not occur in nature, the important point to
discover is whether there is really any similarity of odor
in the several plants of diverse groups that are some-
times utilized by a single species of insect. The facts
alluded to above in regard to the wide variety of selec-
tions made by different species would seem to answer
this question in the negative, as would our own human
sense of odor, which latter may, of course, not be reliable
when dealing with a group of animals so different from
man. We may, I think, rest assured that odor frequently
guides the insects to their food-plant, but we can not be-
lieve that oligophagous or polyphagous species have be-
come accustomed to a variety of plants due to a confusion
of similar odors. There does seem, however, to be one
very striking exception to this among the Pierid butter-
flies. As said before, these butterflies are confined to
Cruciferæ almost exclusively, but one of our species not
infrequently occurs on the garden "nasturtium" (*Tro-
pæolum*). That the pungent taste of this plant is much
like that of a Crucifer is well known and further attested
by the common name, as the true nasturtium of the botan-
ists is a genus of Cruciferæ, while the garden nasturtium
is a Geraniaceous plant.

On account of the very close biological association be-
tween insects and plants in many ways it is true that the
two have been mutually specialized until they have become
highly modified in reference to one another, but this is
not the case with food-plants, as no benefit ordinarily
accrues to the plants and any idea of parallel evolution
must be restricted to a development of undesirable at-
tributes on the part of the plants and adaptations on the
part of the insects to overcome such barriers to feeding.

To avoid these numerous difficulties, it seems clear
that the selection of food-plants by the Lepidopterous
insects so far mentioned, must be considered as depend-
ent upon one or several of a number of factors. Among
these we must include the following:

1. The odor of the plant, and also its taste, which is no doubt closely connected with odor. Associations reasonably placed in this category would be the oligophagous species occurring, for example, on various Cruciferæ, various Umbelliferæ, and various Compositæ. An additional argument for the importance of this factor is seen in the less common utilization by the same insect of several plants in a family like the Solanaceæ[1] where a more or less similar odor does not become a family characteristic.

2. Some attribute of the plant, perhaps an odor but far less pronounced to our own senses than those mentioned above. Species restricted to plants like Leguminosæ or Violaceæ may be considered in this category. Undoubtedly there is some attribute of such plants which insects can recognize in a general way and not as a specific characteristic of some single plant species or genus. The " botanical instinct " of some caterpillars that has frequently been commented upon would appear to be an exaggerated power of recognition of this sort.

3. A similarity in the immediate environment or general form of the food-plant. The effect of something of this sort is seen particularly in oligophagous and also polyphagous caterpillars feeding mainly on trees or shrubs, such as the gipsy-moth, *Cecropia* moth, etc., and those of certain species like some of the Arctiid moths that feed upon a great variety of low plants.

4. Apparently chance associations that have become fixed, whereby diverse plants are utilized by oligophagous species. Secondarily polyphagous species show these in an exaggerated form. On account of their comparatively rare occurrence these seem to be analogous to structural mutations, although they appear to be strictly modifications of instinct. As has been pointed out on a previous page, these are much more apt to occur in some groups (families and genera) than in others.

[8] Possibly in this family, however, the matter may rest upon a physiological basis, on account of the common occurrence of powerful alkaloids in these plants.

THE MANIPULATION AND IDENTIFICATION OF THE FREE–SWIMMING MASTIGOPHORA OF FRESH WATERS

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Not the least among the problems that confront the worker in systematic protozoology is the identification of the minute, free-swimming Mastigophora, representatives of which occur in all cultures, often in great numbers both of individuals and of larger groups. The writer's attempts to find some means of making their identification easier and more certain have resulted in the working out of several methods of dealing with these elusive forms, methods which have been used with success in the laboratory examination of material. They are here presented with the hope that they may be a similar assistance to others.

For the purposes of identification it has been found helpful arbitrarily to divide the Mastigophora into two great groups, on the basis of size; placing those the majority of whose species measure about 12 microns or less along the antero-posterior axis in one group, and those of greater magnitude in the other. It is among the species in the first group that the difficulties in identification seem usually to occur, and it is with this division, therefore, that this paper deals.

The genera included within this first arbitrary division fall within the first four orders of the mastigophora, thus:

SUBPHYLUM MASTIGOPHORA

ORDER MONADIDA
Genera: *Mastigamoeba*
Cercobodo
Cercomonas
Physomonas

333

ORDER HETEROMASTIGIDA

Genera: *Elvirea*
Dinomonas
Pleuromonas
Spiromonas

ORDER PHYTOMASTIGIDA

Genera: *Amphimonas*
Hexamita

ORDER EUGLENIDA

Genera: *Cryptoglena*
Notosolenus

Almost all the mastigophora, but more especially the minute forms, vary in size and shape within the limits of the species. Individuals of apparently the same species are often found half, and even a fourth, of the size which is normal for that species, while abnormally large forms are more rarely encountered. It is the smaller forms which are the most puzzling. These are probably due to: (1) the division of the adults, for binary fission along the line of the antero-posterior axis is the prevailing mode of reproduction among the Mastigophora. Where division is occurring rapidly, in a crowded culture many such forms will be found. Often the young again sub-divide, even before reaching adult proportions. This gives rise to individuals of a species varying widely in size, (2) to the fact that the adults themselves vary in size, even where division is not rapidly taking place, and (3) to the possible production in some forms of swarm spores. Possibly when the life histories of a greater number of the smaller flagellates become known, it will be found that many species multiply not only by binary fission, but by multiple fission as well, either within or without the cyst. It is not unusual to find individuals of a colonial form which have freed themselves, or have been broken away, from the parent community, and which are, temporarily, at least, living an independent

existence.   Adventitious individuals may be derived from such colonial mastigophora as *Spongomonas, Anthophysa, Dendromonas, Uroglena,* and *Ramosonema.*

The following key, which was devised by the writer, has been found helpful in the identification of the minute forms, and in fixing in mind the characters that are the most prominent, and that are useful, under the microscope, in identification.   The key includes practically all the forms of the minute mastigophora that are likely to be found in the waters of bogs, ponds, roadside ditches, creeks, and brooks, and putrefying infusions, in so far as these have been accorded systematic place.   These forms occur over and over again, and their identification has been found to be a matter of less difficulty than was at first thought, before a definite plan for handling them was formulated.

KEY TO THE MINUTE MASTIGOPHORA, THOSE ROUGHLY ABOUT OR BELOW
12 MICRONS

A. Antero-posterior diameter normally about, or less than 8
    microns.
    B. Flagella 2, body kidney-shaped, the smallest of the
       mastigophora ....*Pleuromonas*
    BB. Flagella 3, two trailing, one extended forward.....*Elvirea*
AA. Antero-posterior diameter normally greater than 8
    microns.
    B. With one flagellum.
       C. With greenish chromatophores ....*Cryptoglena*
       CC. Without chromatophores.
          D. Flagellum stiff except at tip ....*Notosolenus*
          DD. Flagellum not stiff, pseudopodia present ....*Mastigamoeba*
    BB. With more than one flagellum.
       C. With 2 flagella.
          D. With one flagellum at each extremity of the
            body ....*Cercomonas*
          DD. Both flagella at same extremity of body.
            E. Body ovate, the anterior extremity the
              smaller ....*Dinomonas*
            EE. Body not normally ovate.
              F. Body spherical ....*Amphimonas*
              FF. Body not spherical.
                 G. Body ribbon-like, twisted ....*Spiromonas*
                 GG. Body not twisted.

H. Body drawn out bluntly poste-
riorly, obliquely truncated an-
teriorly ....*Physomonas*
HH. Body drawn out into an acumi-
nate tip posteriorly, not trun-
cated anteriorly ....*Cercobodo*
CC. Flagella more than 2.
  D. Flagella 3, two trailing, one extended forward ..*Elvirea*[1]
  DD. Flagella four, anteriorly extended; posterior
produced into 2 filamentous appendages....*Hexamita*

In dealing with these minute, free-swimming forms, it
was of first necessity to devise some means of keeping
them quiet and within the field of the microscope when
the higher powers were in use. Several methods both
of retarding movement and of killing were used, but
those which gave the best results were the following.

For the first examination of any sample, a small drop
of the culture was taken and mixed on a slide with a drop
of very viscous gelatine solution,[2] and the whole thor-
oughly stirred together. Or often several drops of the
culture were mixed with an equal part of the gelatine
solution in a watch crystal and used on the slide when
needed. Such a preparation would not keep the pro-
tozoa confined within it alive for more than half an hour,
however, due to the occlusion of the necessary oxygen.

The drop on the slide was now carefully flattened out
and examined without a cover glass under a low power
(16 mm. objective and 10x eyepiece) to ascertain whether
the solution were of a viscosity great enough to check
sufficiently the movements of the flagellates. If not, it
was allowed to concentrate still more by evaporation
until properly viscous, and then covered with a cover
glass. Magnification with the 4 mm. objective and the
8x and 10x eyepieces was found to be great enough for
the identification of most of the forms.

The gelatine used in this method must be of the best
grade and perfectly fresh and clear. It may be slightly

[1] *Elvirea*, because of its variability, is placed both here and in division
AA of the key.

[2] See formulary of reagents at end of paper.

agitated before adding to the culture drop. This includes numerous minute air bubbles, around which the animals may gather and so become concentrated, if the solution is at first thin enough to allow them to make their way through it. It was found that gelatine that had stood ready made up for some time in a warm room became cloudy in appearance and stringy in texture, due to the growth of mould plants and colonies of bacteria.

Another method of retarding the motions of the flagellates, which was partially successful with such minute forms as *Pleuromonas jaculans* and *Elvirea cionae,* was to chill the slide and its water drop thoroughly on a block of ice. This was tried in midsummer, when the sudden reduction in temperature of water that had been quite warm (the culture having stood in the sun) apparently paralyzed the organisms, but they regained their wonted activity after a few minutes' time, since the slide could not very conveniently be kept chilled under the microscope.

The favorite method of the writer for quieting without killing was to utilize a minute aquarium, of a sort that might be used even under the high powers. This was constructed by cutting out a circle of very thin typewriter manifolding paper of good grade and firm texture of slightly less diameter than that of the cover glass, and then cutting from the center of this a concentric aperture about 5 mm. in diameter. This was affixed to the slide with a ring of thin balsam or castor oil, applied with a fine camel's hair brush; the water drop placed in the center, and the cover glass, also ringed with thin balsam or oil, carefully lowered thereon. The oil or balsam sealed the cover glass, and the paper kept it from descending far enough to crush the incarcerated organisms.

At first the flagellates in such an aquarium swim about at their normal rates of speed, but after a time they become stupefied, probably because of the gradual exhaustion of the oxygen supply, and their movements become progressively slower, until finally they cease altogether.

The flagella continue to beat for some little time after the animal has come to rest.

This offers a good opportunity for the observation of the natatory habits, and, as the animals quiet down, for making a closer examination for identification, using higher powers. The smaller the water volume in such a micro-aquarium, the sooner the stupefaction of the confined organisms takes place. The forms studied in the micro-aquarium were usually stained *intra vitam* before their incarceration.

The stains most frequently used for *intra vitam* work were methylene blue (not methyl blue) and methyl green.[3] With each of these a saturated aqueous solution was prepared and diluted to the desired strength.

The stains which were used for killed specimens were methylene blue (not methyl blue), methyl green, gentian violet, and safranin.[4]

The examination of the imprisoned *intra vitam* stained animals has the advantage over the killed and stained ones that it shows the position and action of the flagella, and so leads to a more correct idea of how this should be represented in a drawing. Occasionally one meets with the drawing of a form in which the flagellum is represented as thrown into graceful undulations, whereas, in life, it may be only the tip of that organ that is motile. It was found that frequently the killing reagents caused the flagella to assume unnatural attitudes.

In both the killed and the *intra vitam*-stained animals the flagella takes the stain least of all, often appearing but very faintly, if at all, colored. In *intra vitam* staining, care must be taken not to kill the creatures with too much stain in the attempt to make the flagella stain more deeply.

Killing, in order that examination might be made with the 1.8 mm. objective, using oil, was accomplished by the use of the various well known reagents, such as tannic

[3] See formulary of reagents at end of paper.
[4] See formulary of reagents at end of paper.

acid, osmic acid, acetic acid, formaldehyde, and mercuric chloride solutions.[4]   The best results, however, were obtained by the use of a 1 per cent. aqueous solution of copper sulphate, a reagent which was hit upon in the attempt to find a medium in which death occurred with a minimum of distortion of the flagella.   In nearly all cases the animals very gradually subsided into immobility without any distortion whatever.   A .5 per cent. solution kills them much more gradually.   These solutions must be made up with accuracy, and may be most delicately prepared by counting drops of water and of concentrated copper sulphate solution, as they come from the tip of a finely drawn out pipette.   One hundred drops of water from a pipette the diameter of whose tip measures about 2 mm. makes a sufficient quantity to last for months.

The killing and staining was accomplished in either of two ways, either by killing first and staining afterwards, in which case any of the killing reagents given at the end of the paper was used, followed by the stain, or by performing both operations simultaneously.   This may be done by using strong stains.   The material can be stained either on a slide, or in larger quantities in a watch crystal.   Where the animals were extremely abundant, as they usually were in surface scums or decaying cultures, the latter method was found to be the best, for with the larger quantity of water both the killing reagent and the stain could be more delicately controlled.   Several watch crystals were placed side by side and various gradations of color secured.

The killed specimens were examined at once, and fresh preparations frequently made.   Complete disintegration of these tiny forms takes place a short time after they have been killed.   This is preceded by a distortion of the body.

On the whole, *intra vitam* staining, with the animals retarded in the gelatine solution, or stupefied in the micro-aquarium, gave the best results.   With animals treated thus, the magnifications afforded by the 8x and

10x eyepieces and the 4 mm. objective were found great enough for the majority of the forms.

In the following descriptions of the genera and species the attempt has been made to indicate the characters which are the most prominent ones of the members of each group, those by which the identification can usually be made.   Hitherto less attention than the subject seems to deserve has been given to the manner in which these lower forms make their way through the water—to what may be called their natatory habits.   Many forms show natatory habits which seem to be of a constancy and a distinctness sufficient to warrant their use as character-istics for identification.   This feature has been given at-tention in the following descriptions because it has been found a helpful one in identifying the forms.

It is here suggested that the vibratory motions which some of the smaller flagellates, like *Pleuromonas* and *Elvirea,* exhibit, may be due in part to the influence of pedesis, or Brownian movements.   Carpenter states[5] that all particles suspended in water below $\frac{1}{500}$ of an inch (49 microns) exhibits this phenomenon, and it is a matter of observation that the smaller the particle the more pronounced the vibration.

ORDER MONADIDA

Genus Mastigamoeba Schultze.

Body amœboid, changing shape slowly as pseudopodia are protruded, usually from the sides or posterior por-tion of the body.   Flagellum long, fine, and not easily seen at first in its entirety, because of its rapid motion. These forms move with either a true amœboid motion, or swim by means of the flagellum.   Conn states that in swimming the pseudopodia are retracted, yet we have noticed frequent exceptions in both species.   The swim-ming of these species is clumsy, due perhaps to the ir-regularity of the body.
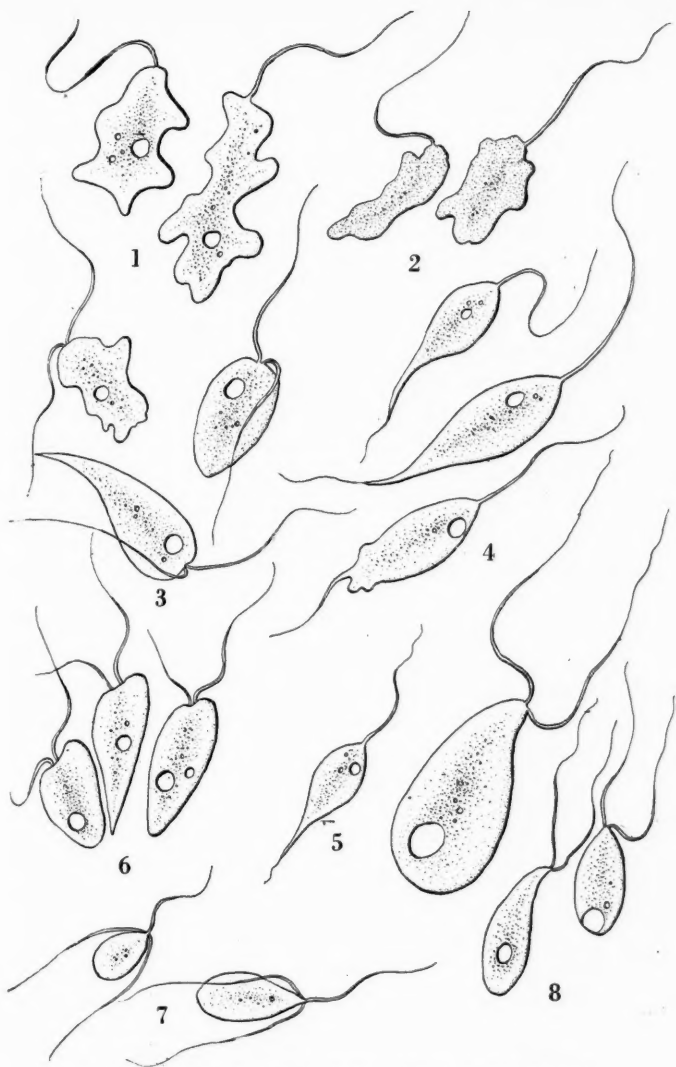
[5] ''The Microscope and Its Revelations.''

FIG. 1. *Mastigamoeba longifilum* Stokes (10–15 μ), 2 individuals.

FIG. 2. *Mastigamoeba repctans* Stokes (8–10 μ), 2 individuals.

FIG. 3. *Cercobodo mutabilis* Kent (8–12 μ), 3 individuals, 1 in the pseudopodia-bearing stage.

FIG. 4. *Cercomonas longicauda* Dujardin (10–13 μ), 3 individuals, 1 protruding pseudopodia at the posterior extremity of the body.

FIG. 5. *Cercomonas crassicauda* Dujardin (6–9 μ), 1 individual of typical form.

FIG. 6. *Physomonas elongata* Stokes (10–13 μ), 3 individuals.

FIG. 7. *Elvirea cionae* Parona (5–8 μ), 2 individuals.

FIG. 8. *Dinomonas vorax* Kent (8–15 μ), 3 individuals.

M. longifilum (Fig. 1) seems to be the most common species in the waters about Ithaca, N. Y., and New Haven, Conn., though *M. repetans* (Fig. 2) was often seen. The former is the larger, the more hyaline, and furthermore possesses at least one quite prominent contractile vacuole. No difference in the length of the flagellum in the two species could be observed.

GENUS CERCOBODO Kraasilstschik.

Body changeable in form from almost globular to elongate, with the posterior extremity usually more or less drawn out, frequently acuminate. This latter form is the one under which the species most frequently appears. The two flagella arise from the anterior end of the body. Often an amœboid form of body is assumed, and locomotion is effected by blunt pseudopodia.

Because of its instability of form, the one species which is the most common has been relegated successively to the genera *Dimastigamœba, Dimorpha,* and *Cercobodo.* The single contractile vacuole is usually present and prominent. The one species observed seems to conform to the *Cercobodo mutabilis* of Stokes (Fig. 3).

GENUS CERCOMONAS Dujardin.

Body globular to ovate, pointed at the anterior and posterior extremities, from each of which arises a long flagellum, the posterior of these being the stouter, a trifle the shorter and less motile. The pseudopodia, which are occasionally produced, are not as well defined as those in the two preceding genera, and are limited to the posterior fourth of the body. These were not frequently seen.

Two species are fairly common: *C. longicauda* (Fig. 4), and *C. crassicauda* (Fig. 5). They may be distinguished by their difference in size.

GENUS PHYSOMONAS Kent.

Body changeable in form, though not possessing pseudopodia, and varying from elongately ovoid to ovoid

pointed at the posterior extremity.   The anterior portion
of the body is normally obliquely truncated, from which
arise two flagella of unequal length.

P. elongata (Fig. 6) is fairly common in all waters of
ponds and bogs, particularly among sphagnum, though
it seems never to occur in crowded cultures.

<div align="center">ORDER HETEROMASTIGIDA</div>

Genus Elvirea Parona.

The body is pear-shaped, and though it may elongate
and contract slightly during swimming, it is quite stable
in form.   Of the three flagella which arise from the ante-
rior extremity, the stouter, shorter one vibrates forward,
and is the organ of locomotion.   The other two trail
behind.

E. cionæ (Fig. 7) is apparently not a very common spe-
cies.   It was found sparsely in the clear cold waters of
springs and creeks.

Genus Dinomonas Kent.

Members of this genus resemble those of the preceding
one in the general shape of the body, but they are larger
and possess but two flagella, both of which extend for-
ward from the more acute anterior extremity.   The con-
tractile vacuole is usually clearly visible and located in
the rounded posterior region.

Fig. 8 was found abundantly in the scums of various
infusions of grasses and leaves, and conforms to the *D.
vorax* of Kent.   *D. tuberculata* (Fig. 9) was often found
in the same infusions with *D. vorax*.   It is possible that
this may be merely another form of the latter species.

Genus Pleuromonas Perty.

Body either kidney-shaped or ovate, the two long fla-
gella arising from a depression in the venter which is not,
however, invariably present.

P. jaculans (Fig. 10) is often very common in stagnant
waters wherever there is decomposing vegetable matter

present. Some infusions will be colored a milky hue
from the multitudes of these forms within them. The
individuals of the species vary considerably in size, some
being no greater than 2 microns along the anterio-
posterior axis. The majority of the individuals, how-
ever, lie within the limits of 3 to 8 microns. They may
be recognized at once by their peculiar agitation and
habit of leaping or jerking about from place to place.
Individuals may sometimes be discovered lying quiet,
except for a gentle vibration—a motion which, it has
been suggested earlier in this paper, may be due to the
influence of pedesis. These quiet forms are to be found
usually near the edges of masses of disintegrating mate-
rial, where they are likely to be overlooked. The bodies
are often particularly hyaline, and the flagella difficult to
make out. Iodine (an alcoholic solution, with potassium
iodide, as given under No. 2, Section B of the Formulary
of Reagents at the end of the paper) as a stain after
killing was found to be the best for these minute forms.
In some cases the flagella could be made to take the stain
well, in others not. Those killed with the stain itself
seemed to be better colored than those stained after
having been killed with some other reagent.

Genus Spiromonas Perty.

Body leaf or ribbon-like, either flattened or more com-
monly twisted spirally, with one or two turns, very vari-
able. Kent says that these forms may even assume an
amœboid form of body. Of the two flagella that arise
from the anterior tip of the body, one trails, one extends
forward and vibrates with great rapidity.

The variable *S. angustata* (Fig. 11) seems to be quite
common in infusions of all kinds, but particularly abun-
dant in those of hay, swamp grasses, and the like.

ORDER PHYTOMASTIGIDA

Genus Amphimonas Dujardin.

Body globular, either attached by a fine posterior pedi-
cel, or free-swimming—the latter being apparently the
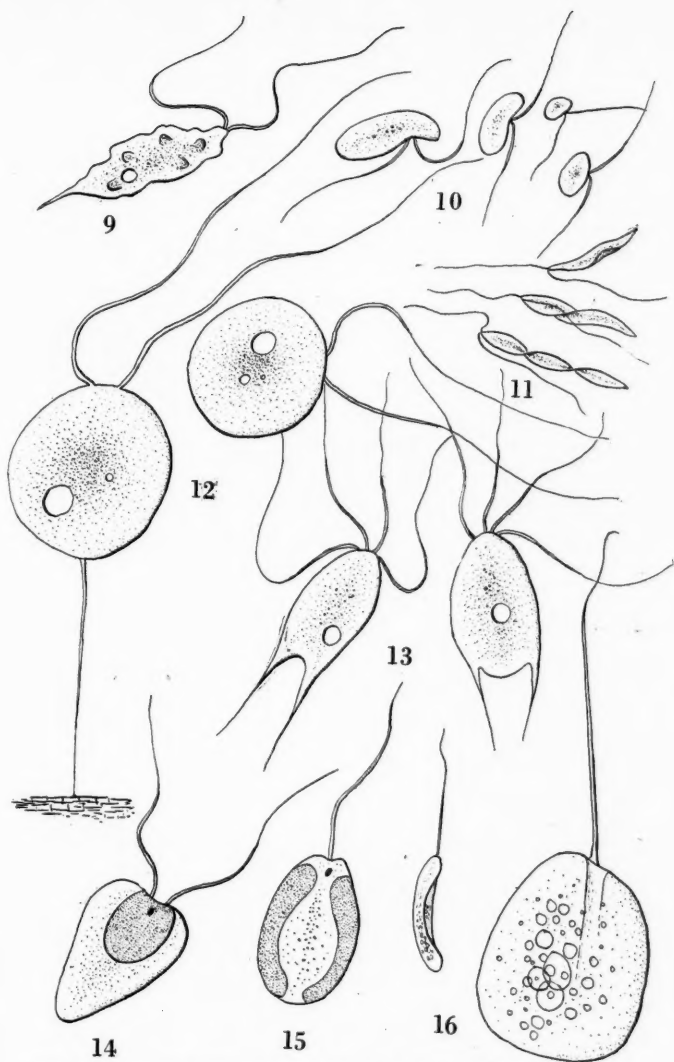
FIG. 9.   *Dinomonas tuberculata* Kent (8–15 μ), 1 individual.
FIG. 10. *Pleuromonas jaculans* Perty (3–9 μ), 4 individuals.
FIG. 11. *Spiromonas angustata* Dujardin (8–10 μ), 3 individuals.
FIG. 12. *Amphimonas globosa* Kent (10–12 μ), 2 individuals, 1 free, 1 attached by a posterior pedicel.
FIG. 13. *Hexamita inflata* Dujardin (10–12 μ), 2 individuals.
FIG. 14. *Cryptoglena conica* Ehrenberg (10–12 μ), 1 individual.
FIG. 15. *Cryptoglena pigra* Ehrenberg (10–12 μ), 1 individual.
FIG. 16. *Notosolenus orbicularis* Stokes (7(?)–15 μ), 2 individuals, 1 turned sidewise to show the concavo-convex shape of the body. This latter form may be, perhaps, another species.

more common condition—by means of two long, fine, equal, anterior, rapidly vibrating flagella. In the free-swimming stage the posterior pedicel is usually absent, though occasionally individuals may be seen trailing after them pedicels of different lengths. These may be pedicels in various stages of retraction into the body.

A. globosa (Fig. 12) seems to be of rather rare occurrence in the waters of ponds and ditches among decaying aquatics.

GENUS HEXAMITA Dujardin.

Body very changeable in form, with two long, filamentous appendages arising from near the posterior extremity of the body. Flagella four, anterior, active.

H. inflata (Fig. 13) was found in water containing *Ceratophyllum* just beginning to decay.

ORDER EUGLENIDA

GENUS CRYPTOGLENA Ehrenberg.

Body oval, not changeable in form, nor varying greatly among the members of the species; possessing one greenish yellow, or greenish brown chromatophore, or two. Flagellum stout at its base, there may be present a red stigma. Swims rapidly, with an undulatory course.

Two species, *C. conica* (Fig. 14) and *C. pigra* (Fig. 15), seem to be quite common in pond waters among such smaller aquatics as *Lemna, Elodea,* etc. They are often found in water in which there is decaying vegetation, also, associated with *Euglena* and *Phacus.*

GENUS NOTOSOLENUS Stokes.

Body hollowed, resembling the bowl of a blunt, thick, wooden spoon; hyaline, colorless, and filled with a large number of large, globular, glassy granules or vacuoles. We have observed but very few individuals which did not contain these. Flagellum long, stiff, and stout, and rigid except that its distal fourth only is motile. This pecu-

liar characteristic affords a good identification character. During swimming it often turns over and over in the water, at which times the concavo-convex shape of the body becomes appreciable.

Either individuals of the species *N. orbicularis* (Fig. 16) vary remarkably in size, or there is more than one species, differing, apparently, only in this particular. The form of the body is, however, constant.

These forms are found, unlike most of the flagellata, most abundantly in fresh waters, particularly in the clear cold waters of springs, that support little plant growth, and they occur usually near the bottom, among the sands and pebbles.

Their progression through the water is often slow and deliberate, and at such times it seems as though the tip of the flagellum were functioning as an exploratory antenna.

FORMULARY OF REAGENTS USED FOR RETARDING, KILLING ANG STAINING

1. Retarding Solutions:
    (1) Gelatine Solution.
        Water .... 5 oz.
        Gelatine .... ¼ oz.
        Heat, to dissolve the gelatine; then allow to cool to the desired viscosity.
    (2) A 1 per cent. aq. sol. of chloretone narcotizes the animals, and gave good results with certain forms, though its manipulation was a trifle difficult.
2. Killing Reagents:
    (1) 25 per cent. aq. sol. tannic acid.
    (2) 5 per cent. aq. sol. acetic acid.
    (3) 10 per cent. aq. sol. mercuric chloride.
    (4) 1 per cent. aq. sol. formalin.
    (5) Invert slide with its suspended drop over the neck of a bottle containing a 2 per cent. sol. of osmic acid. The fumes kill the animals at once.
    (6) 1 per cent. aq. sol. copper sulphate. This gave the best results of any of the killing reagents.
3. Stains:
    A. For *intra vitam* staining. (Make up a quantity of the stain as directed, and then dilute with water to obtain desired depth of color.)

  (1) Sat. aq. sol. methylene blue.
  (2) Sat. aq. sol. methyl green.
  (3) Sat. aq. sol. gentian violet.
  (4) Sat. aq. sol. safranin.

B. For staining after killing, or for killing with the stain itself.
  (Make up a quantity of the stain, and dilute with water for desired color.)

  (1) Sat. alcoholic sol. methylene blue.
  (2) Sat. alcoholic sol. methyl green.
  (3) Sat. alcoholic sol. gentian violet.
  (4) Sat. alcoholic sol. safranin.
  (5) Sat. alcoholic sol. iodine, with 3 per cent. potassium iodide. This gives excellent results. It is a very powerful stain, and must be used in weak solutions.

NEOTENY[1] AND THE SEXUAL PROBLEM

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IT has long been known that the larvæ of certain Uro-
deles sometimes fail to undergo metamorphosis, yet be-
come sexually mature in the larval stage. Perhaps the
best known of such cases of neoteny, as this phenomenon
is called, is that of the Mexican axolotl, long regarded as
a separate species, now known to be an overgrown, sex-
ually mature larva of *Amblystoma tigrinum.* Several
other similar cases have been described, however. All
neotenous amphibians hitherto reported, with the excep-
tion of Allen's (1) thyroidless tadpoles, have been con-
fined to the tailed amphibians, and so far as the writer is
aware, the normal occurrence of precocious ripening of
the sex cells in larval Anura has never been described.
Oddly enough, it is the normal thing, and its occurrence
throws considerable light upon the obscure problem of
sex differentiation and development in the Anura, which
has long puzzled investigators of this subject. It will be
recalled that Pflüger (5) reported years ago, that there
occurs normally in newly metamorphosed frogs three
kinds of individuals, males, females and hermaphrodites,
the two latter forms much more numerous in early stages
than the males. In the course of further development the
hermaphrodites become either definitely male or female,
as the sex ratio for adult frogs is approximately 50–50.
The investigations of R. Hertwig, Kuschakewitsch and
Witschi (2) not only confirmed Pflüger's work, but ex-

[1] The choice of the word neoteny is perhaps not a fortunate one but be-
cause it has come to be associated with the attainment of sexual maturity in
the larval stage, it will be employed here in that sense. Literally, neoteny
means the prolongation or extension of the period of youth, and it has no
necessary relation to sexual conditions.

tended it by showing that anurans apparently first develop solely as females and sexual intermediates, the males only later differentiating from the females and hermaphroditic forms. Moreover, these investigators described in great detail modification of the sex ratios by environmental changes such as extremes of temperature and late fertilization. All of these alleged facts have given rise to the belief that anurans in their sexual development differ greatly from other vertebrates.

For several years the writer has been engaged in studying the germ cells of anurans, more especially *Rana catesbiana,* with the object of testing the theories of sex differentiation and development of the Pflüger-Hertwig-Kuschakewitsch school of German investigators. Although at first inclined to admit their contentions, a more careful survey of my material revealed several facts irreconcilable with their views, but which could not be satisfactorily interpreted. Fortunately an opportunity presented itself of working with Professor E. G. Conklin who suggested, after examining my material, that I was probably dealing with a case of precocious ripening of the germ cells in anuran larvæ, *i.e.,* condition stimulating neoteny, using this word in the sense applied above. The suggestion proved correct, and it is a pleasure to acknowledge my indebtedness to Professor Conklin for giving the clue to correct interpretation of the problem and for many other helpful suggestions as well. The present paper is a brief summary of a more extensive and detailed investigation scheduled for later publication.

In *Rana catesbiana* the larval period is very long, some few individuals requiring four seasons to complete metamorphosis, though the usual period is about two years. The sex of larvæ 55–65 mm. in length is not difficult to determine by examination of the gross structure of the gonads, but if such superficial examination is supplemented by a hasty survey of the microscopic appearance of the germ cells, then oddly enough hopeless confusion of the sexes results, and what were apparently males from macroscopic evidence turn out to be apparent fe-

males. The sex ratios will vary greatly according to the stress laid by the observer on the gross appearance of the gonads (and it must be admitted that in early stages the gonads of the two sexes are remarkably alike) or upon the cytological evidence as it has heretofore been interpreted. In larvæ of 80–100 mm. length the sex ratio is approximately 50–50 when based on the evidence presented by the gross appearance of the gonads; on the other hand, the cytological criterion, as it has been interpreted, practically does away with males, while most of the animals are apparently female. It is probably due to this erroneous interpretation of cytological conditions that such confusion reigns in the literature regarding sex in anurans.

The germ cells of larvæ 45 mm. and over, both *male* and female, are found in early maturation stages. Such animals are about 8 months of age. Practically all of the female cells are in the leptotene or pachytene stage. In the females the leptotene and pachytene stages do not persist for any length of time, but give place to the period of growth, in which the cells with pachytene nuclei increase greatly in volume, are invested by a follicle of peritoneal cells, and become typical oocytes. Those cells bordering the lumen of the gonad, are first to enter the growth period, and by reason of their great increase in size, fill up the cavity. Around the periphery of the gland a ring of cells with leptotene and pachytene nuclei persists, giving rise later to oocytes of a younger generation. Scattered through the gland are a few oogonia with polymorphic nuclei.

The female gland increases greatly in size due to the growth of the oocytes, becomes much infolded and convoluted by inequalities of growth, thus taking on the characteristics of the typical ovary of the adult. These typical ovaries are to be found in larvæ over 80 mm. long. The gonads probably persist in this condition for several years after metamorphosis, the oocytes growing very slowly. According to the observations of Hertwig and others, the females of *Rana temporaria* and *Rana escu-*

lenta do not become fully mature and ready for copula-
tion until the fifth season after metamorphosis. The
writer has captured two females of *Rana catesbiana* two
seasons after metamorphosis which were yet sexually
immature, hence it seems that the females of this species
also require a long period of time in which to develop
sexual maturity. The developmental history of the male
gonads and germ cells is quite different, and when rightly
interpreted fails to show female animals transforming
into males and vice versa, or abnormal sex ratios.

It was stated that germ cells of the male larvæ begin
their maturation cycle simultaneously with those of the
females. This is a very unusual condition and probably
unique among the vertebrates though common enough
in the invertebrates. It will be recalled that in the verte-
brates—for example the mammalia, a very long period
of time, sometimes years, separates the maturation cycle
of the sexes. In many instances in the female, the initial
maturation changes preceding the growth period of the
oocyte, occur before birth, whereas the same nuclear
changes in the male cells do not make their appearance
until shortly before the attainment of sexual maturity.
It has become the custom for investigators of sexual con-
ditions in the Anura to use this fact of the early occur-
rence of maturation stages preceding the growth period
of the oocyte in the female as a cytological criterion for
differentiating the sexes in the larval stages. Unfortu-
nately this principle, though true enough for other verte-
brates is not applicable to Anurans and the result has
been hopeless confusion of the sexes because in this group
maturation occurs in larval males.

From the period of formation of pachytene nuclei, the
history of the sexes is quite different in *Rana catesbiana*
and unmistakable if a complete series of larval stages is
obtained. In justice to other investigators whose results
the writer criticizes as based upon misinterpretation of
sexual conditions, it is fair to point out that of all exist-
ing species of Anura, *Rana catesbiana* is apparently the
only one in which precocious ripening of the male germ

cells goes as far as the formation of the maturation division in first year larvæ, and ripe spermatozoa in second year animals. This is of course due to the extraordinarily long larval period. In *Rana temporaria* and *Rana esculenta* the larval maturation changes apparently go only up to and including the pachytene stages before degeneration sets in. In Bufo, the precocious ripening of the sex cells is confined entirely to the cells of Bidder's organ and continues up to the pachytene stage before growth begins.

The male larvæ of *Rana catesbiana* undergo two distinct seasonal maturation cycles as larvæ. The first occurs in young animals 45–60 mm. total length, despite the fact that the germ gland is in an extremely undifferentiated condition. The germ cells develop normally through the leptotene, pachytene, diplotene and tetrad formation stages, but invariably degenerate and go to pieces during the late metaphase or early anaphase of the first maturation division. The centrosomes fragment and the spindle apparatus is aberrant. There are no second maturation divisions, though occasional giant spermatid-like structures may form by the growth of axial filaments from the centrosomes of first spermatocytes. The cells of the first larval maturation cycle degenerate. Through active mitotic division the few primary spermatogonia scattered throughout the gland give origin to those cells which later undergo the second larval sexual cycle. This second cycle occurs near the end of larval life, *i.e.*, usually about two years after hatching. Oddly enough the second maturation cycle is normal, and gives rise to functional spermatozoa in the larvæ, though the efferent ducts of the testes are not yet fully formed. The germ cells and tetrads of the first sexual cycle are aberrant in size and character, those of the second cycle are normal in every way.

The diploid chromosome number of the larvæ is twenty-eight, the haploid number is fourteen. There is no evidence of an accessory chromosome.

Probably the larval sexual cycle just mentioned is an interesting example of a "carrying over" in ontogeny of an earlier phylogenetic condition when the Salientia were sexually mature and normally reproduced as larvæ. It is interesting to note in this connection that male anuran larvæ whose period of metamorphosis is indefinitely postponed, as for example by thyroid extirpation, readily mature sexually, in so far as the production of ripe spermatozoa is concerned.

The male germ cells, unlike those of the female, do not undergo growth, except in relatively rare instances to be described later, and consequently do not fill up the lumen of the gonad. This lumen later is obliterated by the migration into the gonads of cells from the mesentery and possibly from the cortical substance of the adrenal gland. From this ingrowth the testicular interstitium and rete apparatus develops. The efferent tubules at the time of metamorphosis form a connection with the mesonephros. The true sex cords of the testis arise as proliferations of the germinal epithelium, and not as so often claimed for amphibia, as ingrowths from the mesonephros.

This phenomenon of precocious ripening of the male germ cells of *Rana catesbiana* larvæ undoubtedly occurs in other Anura, though is not carried so far as in the bullfrog. The figures of Kuschakewitsch and Witschi show clearly that this condition exists in *Rana esculenta* and *Rana temporaria*. Indeed, it seems more than likely that these writers have mistaken male frog larvæ whose germ cells were in early pseudo-reduction stages, for hermaphrodites and females. The so-called sexually indifferent or sexually intermediate forms of the Pflüger-Hertwig school are very probably male animals whose germ cells show precocious ripening as far as the pachytene stage. This is plainly evident from their photographs, drawings and descriptions. This probable misinterpretation of the cytological data accounts for the transformation of such so-called hermaphrodites into male animals, so minutely described by these investigators. Using the chief cri-

terion of sex differentiation employed by Witschi, *i. e.*,
that all germ cells in the larval gonads showing pseudo-
reduction (leptotene and pachytene stages) are to be re-
garded as female, the writer obtains in his set of 2,000
animals, 96 per cent. females and only 4 per cent. males.
In larvæ of 85 mm. length the percentage of males is zero
if this criterion of sexual differentiation is employed.
The writer has found so far no evidence that the sex
ratios of Anure are any different from those of other
vertebrates, and is inclined to regard the confusion con-
cerning sex differentiation and development in anurans
as a result of interpretating male animals showing pre-
cocious maturation changes of the germ cells as females
and hermaphrodites. Sex in the frog does not appear
to be nearly so labile and easily influenced as some in-
vestigators claim. Professor Hertwig's "late fertiliza-
tion" experiments are more satisfactorily interpreted on
the chromosomal hypothesis of sex determination, than
on any other.

One reason so many workers dealing with anurans
have regarded these animals as possessing hermaph-
roditic tendencies is the occurrence of "oocytes," so-
called, in the testes of larval and adult frogs and the
presence of the peculiar ovary-like structure, the organ
of Bidder, in the Bufonidæ. The origin of these apparent
oocytes in *Rana catesbiana* has not yet been worked out
as completely as the writer could wish; however, enough
data has been collected to warrant a tentative explana-
tion of their occurrence in this form and the same data is
suggestive as regards the nature of Bidder's organ in
the male toad, at least suggestive enough to warrant a
reinvestigation of this structure, now generally regarded
as a rudimentary ovary.

In male *Rana catesbiana* larvæ, these large oocyte-
like cells are of frequent occurrence, and assume this
character while in the pachytene stage. Previous to the
growth period they are indistinguishable from the other
pachytene male cells of the gonad. During the growth
stage, which is later followed by their degeneration and

disappearance, they are similar in every way to the cells of Bidder's organ. Their follicles are derived from surrounding peritoneal or stroma cells. These follicles are commonly observed surrounding isolated spermatogonia. In certain male gonads a few cells grow to the size of oocytes, and possess yolk nuclei. The presence of yolk, however, is no sex criterion for the male germ cells of many animals form yolk as for instance *Ascaris,* and the apyrene spermatozoa of certain Prosobranchs, and the degenerating cells of the frog.

The larval spermatocytes of the first maturation cycle are in many instances of enormous proportions, scarcely smaller than many organs of Bidder cells near the end of their cycle. It is not impossible that there may be a genetic relation between these two types of testicular elements. This question is reserved for further discussion in a later paper.

The writer is of the opinion that these "oocytes" are of the same nature as the cells of Bidder's organ in *Bufo*. It might be suggested as a possibility worthy of consideration, that in male animals such cells may be of true male character, but owing to the precocious sexual cycle, itself a vestige of a primitive phylogenetic condition when the Anura were sexually mature in the larval form, a few of the germ cells are unable to complete their cycle, and simply grow to an abnormal size, thus assuming the unspecialized character associated with oocytes. These cells degenerate during the second larval maturation cycle when normal sex products are produced. It is rather significant that the whole first larval sexual cycle is abortive in almost every feature. For instance the spermatocytes are abnormally large, the tetrads equally so, the first maturation division never proceeds past the anaphase, the centrosomes fragment, form polyasters, and sometimes axial filaments. Entire cysts of perfectly formed spermatocytes go to pieces in the very act of division, and most of the germinal elements show marked evidences of a deep seated protoplasmic disorganization. In view of these facts it is possible that the cells of

Bidder's organ in *Bufo,* and the oocytes-like cells of
anurans may not be true oocytes, despite their appear-
ance, but may be merely senescent cells, occurring in the
course of an abortive and degenerate larval sexual cycle.
Bidder's organ on this assumption is the vestigial remains
of a primitive sex gland functional when the Bufonidæ
reproduced in the larval stage. The functional gonads
of present day toads represent recently acquired struc-
tures superimposed upon the phylogenetically older and
degenerate glands.

In *Rana catesbiana,* a more primitive anuran type
than *Bufo,* the entire larval male gonad might with some
plausibility be compared to an organ of Bidder in which
only a few cells assume the oocyte character whereas the
remainder develop a little further, *i.e.,* to the first matura-
tion anaphase, when they too go to pieces. The writer
suggests this view of Bidder's organ tentatively, and
pending further investigation does not regard himself as
irrevocably committed to it. The facts are suggestive,
and that is all that can be said at present.

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SHORTER ARTICLES AND DISCUSSION

THE TABULATION OF FACTORIAL VALUES[1]

In *Science* for January 23[2] Dr. Ellis L. Michael discusses the validity of the ordinary system of tabulation in the determination of the probable number of bacteria in an emulsion. He argues in favor of the use of the logarithms of the measurements instead of the direct measurements because the former give a symmetrical distribution, while the latter give one that is distinctly asymmetrical. As Dr. Michael has invited discussion it may be of interest to mention briefly a similar method used during the last two years in a study of the germinal and environmental factors affecting eye facet number in the bar races of *Drosophila*. A report of the method was made at the St. Louis meeting of the American Society of Zoologists and the results of its application to the particular problems in hand are being published in a series of papers.[3]

In working up the data it became evident that the demands of the biological analysis were not adequately met by the system of arrangement in classes with equal facet numbers. The wide range in individual stocks and the still wider differences between different races made it desirable to express relations directly in terms of factorial units affecting facet number rather than in facet numbers. In dealing with a stock averaging 30 facets as compared with one averaging 300 facets it became evident that a one facet change at the mean in a 30 facet stock represents the same factorial value as a ten facet change at the mean in a 300 facet stock and that *a corresponding principle applies within the range of a single stock*. Accordingly the classes were arranged

[1] Contribution from the Zoological Laboratory of the University of Illinois, No. 152.

[2] "Concerning Application of the Probable Error in Cases of Extremely Asymmetrical Frequency Curves," *Science*, N. S., 51: 89–91.

[3] "A Change in the Bar Gene of *Drosophila* Involving Further Decrease in Facet Number and Increase in Dominance," *J. Gen. Physiol.*, 1919, 2: 69–71. *J. Exp. Zool.*, 1920, 30: 293–324.

so that the facet range of each class is a fixed per cent. of the mean facet value of *its class*. In other words the class facet ranges vary in such a way as to give the same logarithmic range to each class.

As an illustration eye facet counts in 488 females of the unselected white bar stock may be taken. The following table gives the frequency distribution obtained when the classes have the same facet ranges:

Facet Counts	Frequency in Per Cents.
16– 21	0.2
22– 27	0.2
28– 33	2.9
34– 39	10.9
40– 45	14.3
46– 51	12.3
52– 57	12.9
58– 63	11.7
64– 69	9.2
70– 75	8.0
76– 81	3.9
82– 87	3.9
88– 93	3.3
94– 99	2.9
100–105	1.4
106–111	1.6
112–117	0.2
118–123	0.0
124–129	0.0
130–135	0.2

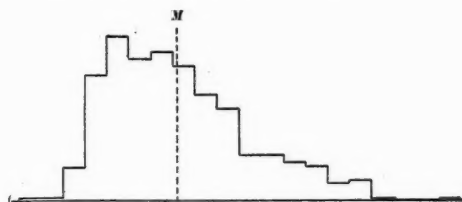The same arrangement is shown in graphic form in the following figure:

FIG. 1.

There is a marked positive skewness. The next table uses the same original data but with the facet range in any class equal to ten per cent of the mean of *that* class:

Facet Counts	Frequencies in Per Cents.	Factorial Units from Mean
20– 21	0.2	— 9.93
22– 23	0.0	— 8.93
24– 26	0.2	— 7.93
27– 29	0.6	— 6.93
30– 32	1.8	— 5.93
33– 35	3.1	— 4.93
36– 39	8.2	— 3.93
40– 43	9.6	— 2.93
44– 48	11.1	— 1.93
49– 53	11.9	— 0.93
54– 59	11.1	+ 0.07
60– 65	11.3	+ 1.07
66– 72	10.4	+ 2.07
73– 80	5.9	+ 3.07
81– 88	5.1	+ 4.07
89– 97	5.5	+ 5.07
98–107	2.3	+ 6.07
108–118	1.4	+ 7.07
119–131	0.0	+ 8.07
132–145	0.2	+ 9.07

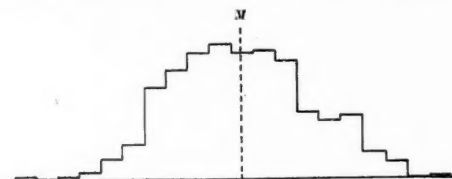The following figure is based on the same arrangement:

Fig. 2.

This is much closer to a normal distribution of frequencies than in the ordinary method. It is correspondingly more reliable in the determination of the various constants.

If the biological assumption upon which this tabulation is based is correct the classes are of equal value as far as the *factors* affecting facet numbers are concerned though the facet ranges are different. In following out this view the intervals on the variation scale have been expressed in terms of class units, each unit being equivalent to a factor which produces a change of ten per cent. in facet number. Some arbitrary point, for instance the mean of the unselected stock, may be taken as the point of reference or zero and every facet value has a corresponding factorial value on the scale. The variation constants may be ob-

tained in the ordinary way but in terms of factorial units and not facet units. The standard deviations are used directly as coefficients of variation.

The biological validity of the factorial method as given is of course dependent upon the correctness of the view that eye facet numbers have such a relation to environmental and germinal factors as is indicated. The normality of the factorial distribution has already been mentioned. General embryological considerations favor proportionate action of factors rather than action by accretion. But I wish to mention particularly the definite experimental proof that at least one factor, temperature, is in agreement with the hypothesis. Seyster[4] has shown that in bar eye facet number decreases with increase in the temperature at which the larvæ of *Drosophila* are reared. This decrease follows van't Hoff's law if an inhibitor of facet number is assumed as the effective agent upon which the temperature acts. Krafka[5] has demonstrated that this general law applies to ultra-bar as well as to bar eye and that for the different bar stocks the effect of a degree of change in temperature is roughly proportional to the mean value of the stock and the same is approximately true for the effects of a degree of change in temperature throughout the range of a single stock. The following table gives the facet values for ultra-bar, low selected bar and unselected bar at 15° and 25°:

15° Facet Values	25° Facet Values	Differences	Ratios of Differences
51.5	25.2	26.3	1.0
189.0	74.2	114.8	4.4
269.8	120.5	149.3	5.7

Representing the effect of a ten-degree difference for ultra-bar as unity, low selected bar has 4.4 times and unselected bar 5.7 times this difference. It is obvious that difference in facet number is not a good measure of the value of the temperature factor.

On the other hand, if facet values are reduced to factorial values according to the method given above the results are as follows:

4 Seyster, E. W., ''Eye Facet Number as Influenced by Temperature in the Bar-eyed Mutant of *Drosophila melanogaster (ampelophila)*,'' *Biol. Bull.*, 1919, 37: 168–182.

5 Krafka, Joseph, Jr., ''The Effect of Temperature upon Facet Number in the Bar-eyed Mutant of *Drosophila*,'' *J. Gen. Physiol.*, 1920. (In press.)

15° Factorial Values	25° Factorial Values	Differences	Ratios of Differences
− 0.83	−7.86	7.03	1.0
+12.17	+2.79	9.38	1.3
+15.72	+7.73	7.99	1.1

This is a much closer approach to unity for the ratios than in the case of facet values and the units employed may be taken as fairly close measures of the temperature factor.

A change of one facet is therefore not of equal factorial value at different points on the variation scale as far as temperature is concerned. A plotting of the data using facets as the units does not give a uniform factorial scale. Suppose temperature to be the only factor causing variation in the facet number of a particular stock but knowledge of the actual temperatures involved in the production of a particular population to be lacking and it is desired to derive the value of the temperatures from the facet values. Obviously the closer approximation is obtained by the tabulation in which each class has a facet range equal to a definite per cent. of its facet mean. Krafka's data show that even in this case the determination is not exact but certainly the error is of a much lower order than that involved in using facets as the units.

<div align="right">CHARLES ZELENY</div>

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AN EXPERIMENT ON REGULATION IN PLANTS[1]

IT is a fundamental fact that of the enormous number of buds on a tree only a few of these normally develop into branches. Every bud, however, has the capability of growth and will grow into a branch if the more apical bud or buds are removed. Even normally, in uninjured trees, some of the lateral buds grow into

[1] After this paper was written, my attention was called to an article by Child and Bellamy (*Science*, N. S., L, 362, 1919), in which somewhat similar experiments were reported and the same conclusion arrived at. Physiological isolation of two regions of a whole plant was produced by low temperature instead of by actual killing of tissue as in my experiments. In view of the importance of growth phenomena I believe it worth while to again call attention to the conclusions to be drawn from these facts, especially as the experiments of Child and Bellamy refer only to the influence of a growing stem on the growth of other stems and not to the influence of growing roots on the development of roots in other regions of a plant.

branches and the characteristic form and type of growth of a plant are thus determined.   It is a species characteristic.

An analysis of the factors which retard the growth of lateral buds can best be made on plants with only a few buds, and an excellent discussion of the problem has been given by McCallum.[2] McCallum worked with the scarlet runner bean, *Phaseolus multiflorus*.   The cotyledons of this bean remain at the surface of the ground and the buds in their axils never develop unless the growing stem is injured or removed.   Then they invariably develop and form shoots.   No amount of wounding short of removal of the growing tip will cause these buds to grow.   They never grow if the terminal bud is present, no matter how much food or water is available with optimum light and temperature conditions, and they always start to grow if the terminal bud is removed and at the same time the plant is starved by cutting off cotyledons and root systems or is practically dehydrated by placing it in a very dry atmosphere.

To describe the phenomenon we say that the tip inhibits the growth of buds below it.   Only a *growing* tip has this inhibitive action, for McCallum showed that if the tip is kept in a hydrogen atmosphere, which prevents its growth, the cotyledonary buds begin to grow.   Later if the tip is removed from the hydrogen to air, it also will again grow.

The inhibitory influence of a growing stem tip on latent buds is exerted only downward.   A growing root exerts an inhibitory influence on the development of roots above it and this influence passes upward along the stem.   If the roots of a bean plant are removed, new roots will develop along the stem wherever there is most moisture.   The new roots do not come from root buds as the new shoots come from shoot buds, but they arise from unformed regions of the stem.   The inhibitory influence on root formation which passes upward moves along the vascular bundles and is restricted to that section of the stem immediately above in line with the point of injury to the root.   This, if the stem of a bean plant is kept moist and a small notch is made (so as to cut the vascular bundle) in the stem below this moist region, the inhibitory action of the main roots will be cut off by the notch and secondary roots can now form in this moist region only on the side above the notch (see Fig. 10, p. 116 of McCallum's paper).

The inhibitory action of a growing tip in the bean on buds

[2] McCallum, *Bot. Gaz.*, XL, 97 and 241, 1905.

below is not localized in the stem perhaps we may say is not transmitted in a direct line in the stem. A notch cut half-way across the stem will not cause the cotyledonary but directly below it to grow. In *Bryophyllum*, however, Loeb's[3] experiments have indicated that the inhibitory influence of a leaf on the growth of axillary buds passes downward in the same sector of stem as the leaf itself. Moreover the inhibitory influence appears to be of the nature of material flowing, because the pathway of the inhibitory influence is affected by gravity. This is illustrated in Figs. 11, 12 and 13, pp. 349 and 351 of Loeb's paper. This influence of gravity is a fundamental fact whose importance for the explanation of regulation phenomena must not be overlooked.

It is obvious to one who seriously contemplates the facts of regulation that the influence of one part over another in the organism must be either similar to nerve influence and depend on living protoplasmic continuity between the parts, or due to the actual transport of material from one region to another. Unless we are to assume the existence of a guiding all powerful form-determining spirit or force, which is as difficult to prove as to disprove, there is no other than these two explanations. These we know to be two means of "action at a distance" in animals and we might expect them to be operating in a plant also.

Let us consider the second of these possibilities—transport of material. Two views are prevalent regarding the nature of this transport. (1) A growing stem may be supposed to form material which inhibits shoot formation and a growing root to form material which inhibits root formation.[4] These special inhibition substances pass downward and upward in the stem respectively. Polarity is a direct consequence of the formation of these substances and their direction of flow. Loeb has pointed out several instances of growing roots inhibiting the formation

[3] Loeb, J., *Journ. Gen. Physiol.*, I, p. 337 and 687, 1919.

[4] It has been suggested that a growing stem forms material which inhibits shoot formation but favors root formation. The root formative substances collect at the basal end of a cut stem and induce the formation of roots there. That this assumption will not hold is indicated by McCallum's experiments on root formation already mentioned. (Fig. 10, p. 116.) The stem of a whole bean plant is surrounded near its upper end by water held in a glass vessel. This gives favorable conditions for the development of roots but none grow so long as the roots of the plants are intact. If they are cut off new roots form, not at the base of the stem where the cut is and where root forming substances should collect, but in the water high up on the stem.

of roots in other regions of a plant of *Bryophyllum* and of even stopping the further growth of roots which had started to grow.

(2) In a whole plant, because of a certain morphological structure, the nutrient channels are such as to carry food material to growing regions. A plant grows at both ends and so long as these are intact and growing food flows toward them. If removed, food flows to other points and starts the growth of dormant short buds or root primordia. Once a stem or root has started growing Loeb's[5] experiments show very clearly that the mass of growth formed is proportional to the mass of materials available.

I believe that light is thrown on this problem by some experiments which I performed in 1910, while a student at Columbia University, and repeated at Princeton in 1912, but which have never been published. They are designed to divide a plant into two parts physiologically but not morphologically. A jet of steam was directed against the stem of a young bean plant between cotyledons and first pair of leaves in order to kill the tissue throughout the stem in this region. In some plants the leaves and growing tip above this region wilt and die but in many cases not only does no wilting occur but the tip continues to grow and *as rapidly or more rapidly* than control plants under the same conditions which are unsteamed. Nevertheless the *cotyledonary buds below the steamed region begin to grow and roots start to appear just above the steamed region.* If the air were sufficiently moist or the region surrounded by water there is no reason why these incipient roots should not grow out into a typ-
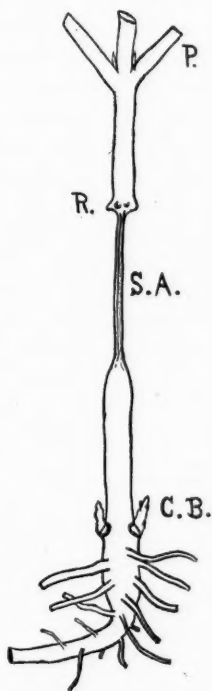
FIG. 1. Drawing showing condition of a bean plant eight days after the stem has been steamed. The top and tap root have been cut off to reduce its size for preservation. They were alive and growing during the experiment. Note that the cotyledonary buds (C. B.) grow below the steamed area (S. A.), and roots (R) appear above the steamed area. P, petioles of first pair of leaves.

[5] Loeb, J., *Journ. Gen. Physiol.*, I, p. 81, 1918.

ical root system. I have preserved the stems of plants steamed in this way and Fig. 1 is a drawing of one of these. It will be noted that the steamed region, which was exposed for three minutes in this plant, has shrivelled to a hard woody connection about 24 mm. long. Table I gives the data regarding the growth of control and steamed plants whose tops were not killed by the steaming. The average growth for the three controls is 40.5 mm., and for the steamed plants, 59 mm. It is evident from the table also that the terminal bud has grown in the 24 hrs. immediately after steaming so that we cannot say that the steaming caused even a temporary cessation of growth of the tip.

TABLE I

RATE OF GROWTH OF STEAMED AND CONTROL BEAN PLANTS

	Plant	Time steamed in Seconds	Length of Shrivelled Stem in Mm.	Mar. 7, Length of 1st Internode Above Leaves	Mar. 8, Length of 1st Internode Above Leaves	Mar. 11, Length of Internode		Mar. 15, Length of Internode		Total Growth in 8 Days, in Mm.
						1st	2d	1st	2d	
Controls, unsteamed.	1			1.5	1.5	7.5		26.0		24.5
Cotyledonary buds	2			4.0	6.0	16.5	3.5	25.0	14.0	35.0
do not grow.	3			5.0	10.0	34.0	2.0	50.0	17.0	62.0
	1	5	8.0	2.0	5.0	20.0		38.0		36.0
March 7, 1912.	2	7	5.5	5.0	7.5	9.0		21.0		15.5
Steamed. Cotyle-	3	10	9.5	7.0	11.0	39.0		69.0	11.0	73.0
donary buds all	4	15	15.0	14.0	24.0	60.0	14.0	66.0	47.0	99.0
grow.	5	20	14.0	5.0	8.0	37.0		71.0	20.0	86.0
	6	180	24.0	13.0	18.0	47.0		48.0	10.0	45.0

It is certainly true that sap must pass up the stem of these steamed plants, otherwise the tops would remain turgid and growth occur. Root inhibiting substances, if formed, must have passed upward along with the sap. Nevertheless we find roots developing above the steamed region despite the fact that the plant has a normal living root system below. The evidence is conclusively against the existence of definite root inhibitive substances. If sap can pass upward in a steamed area we might expect that it could pass downward also. If inhibitive substances are formed by a growing stem these materials should reach the cotyledonary buds below. Nevertheless these buds develop. Since we can not necessarily argue that because material can pass up a stem it must also pass down, the evidence points against the existence of shoot inhibitive substances, but is not unequivocal.

In a plant which has been steamed the nutrient channels are the same as in a normal plant.   The apical bud is growing and attracting material to it so that we cannot say this food material is now available for the cotyledonary buds as we might had the growing tip been actually cut off or prevented from actively growing by a hydrogen atmosphere.   The evidence is conclusively against the view that growing points prevent the growth of dormant buds by attracting and utilizing the nutrient material.

It would seem that the inhibitive influence must be dependent on living functioning protoplasmic connections.   How are we to conceive of an influence of this sort without invoking a vitalistic explanation?   I believe the explanation lies in the direction developed at length with the aid of metal models by Lillie.[6]   Growing points are of a different electrical potential as compared with other points and the currents so generated passing through dormant buds in the proper direction, prevent their growth.   The potentials are phase boundary or membrane potentials, possibly dependent on selective ionic permeability or solubility of two phases (cell and medium) to ions, and consequently dependent or normal permeability conditions throughout the plant.   Interruption of living protoplasmic connections, then, means merely the interruption in continuity of semipermeable membranes in longitudinal axes of the plant (vascular bundles?).   While we may be sure that the steamed portion of a plant will conduct an electrical current, since its normal semipermeable membranes have been destroyed there is no means of obtaining a return circuit.   The plant is divided into two electrical systems instead of one and behaves practically as two distinct plants.   As Lillie[7] has suggested the effect of gravity on the inhibitive influence of growing stems, pointed out by Loeb, may be explained by movement of sap downward and passage of a greater current through this region because of increased electrical conductivity there.   Biological polarity thus becomes electrical polarity and a given process at one region or pole is automatically accompanied by the reverse process at the opposite region or pole.

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[6] Lillie, R. S., *Biol. Bull.*, XXXIII, 135, 1917.
[7] Private communication.

INHERITED PREDISPOSITION FOR A BACTERIAL DISEASE

As soon as it can be demonstrated that in a process under investigation a given factor has a very marked influence, this factor is more often than not looked upon as the sole cause of what happens. It is indeed very difficult not to overemphasize the importance of a new link in a chain of causes, which has been hitherto overlooked, and which one is fortunate enough to discover. To give a few instances from a field familiar to us, we can cite three factors in the evolution of species which have each by one author been elevated to the rank of "the" cause of species formation. Natural selection was the cause of evolution in the eyes of Weismann, and every other factor was looked upon as subordinate. In the same way Wagner overemphasized the importance of isolation, and de Vries would have us believe that mutation was the main, if not the sole, cause of evolution. The greatness of Charles Darwin lies in the fact, that he was not led away from a consideration of all the possible factors by the temptation to pad out the importance of any one link in the chain of causes.

In a few instances the discovery of a new and very important factor in the causation of a process or set of phenomena sets all the investigators working in the new field just opened up. And often the attention is unduly taken away from other causes. In pathology the discovery of the rôle which microorganisms play in the causation of certain diseases has resulted in the almost absolute neglect of the study of possible other factors in the causation of these same diseases.

In the illness of an individual, infection by a specific microorganism is a very important factor in certain cases. But it is clear that, besides this infection, other factors influencing the qualities of the subject can be of great importance. Very often we find that, besides the presence of the specific organism, predisposing factors play an important rôle, such as the simultaneous presence of another infection (tuberculosis after measles) special conditions (diabetes, possibly beri-beri) ; causes lowering the vitality (exhaustion, inanition).

Besides factors of the environment, which in themselves are not pathogenic factors, it is evident that factors given in the composition of the individual, inherited factors, can cooperate in the causation of disease.

To make the statement general, we can say that illness is a condition caused by the cooperation of a series of factors, of which some are genetic, heritable, given in the composition of the individual's germ, and others are non-genetic, influencing the individual from the outside. In different combinations of other causes, individual factors can have a very different influence. In certain cases, therefore, different factors can be looked upon as the one which tips the scale, and consequently as "the" pathogenic moment.

The discovery of microorganisms and their rôle in disease has relegated other pathogenic causes to the background, and especially in those diseases where presence of the specific microorganism can always be demonstrated.

In some diseases the presence of a specific microorganism is not demonstrated, and an important non-bacterial factor seems to be the chief determining cause (some cases of carcinoma and of traumatic diabetes). In other cases, presence of a specific microorganism is certain demonstrable, but it seems as if other factors play an important rôle. Tuberculosis is a typical instance. And finally we know diseases, in which it appears as if presence or absence of a specific microorganism constitutes the almost exclusive cause of the difference between affected and healthy individuals (plague).

In the first group, diseases in which microorganisms play no rôle, the factors which cause the abnormal condition can be real environmental factors, but in some instances they are clearly genetic factors, developmental factors transmitted through the germ, genes. We know real hereditary diseases, where an inherited, genotypic peculiarity seems to be the causating factor (hemophily, Huntington's chorea, Daltonism).

In the second group, in those cases, therefore, where predisposition seems to have an influence comparable in its magnitude to infection, this predisposition can have very different causes. In some cases the cause of a predisposition is very clearly non-genetic, environmental (pneumonia after influenza, tuberculosis of the joints after trauma). In other cases, however, inherited constitution is very probably an important factor.

The "inheritance" of tuberculosis has been a point of unending controversy. Very often tuberculosis occurs in families in a way which makes us think of inheritance. According to many authors this occurrence of tuberculosis in families is simply caused by the greatly enhanced chances for a heavy infection. Others

however believe in the possibility of a real inheritance of the disease. It is very evident that the discussion has been very much hampered by a confusion of "inherited" and "congenital." And it has seemed to a great many authorities as if the question as to the existence of an inherited moment in tuberculosis could be answered by an investigation into the possibility of pre-natal infection.

Lastly, there are authors who believe in the inheritance of a certain disposition for tuberculosis.

From the fact that practically all persons above the age of twelve react positively to von Pirquet's test, it can be seen that tuberculosis infection is not as inevitably the cause of tuberculosis, as for instance pneumococcus infection is the cause of septicæmia in the mouse. Every practising physician has seen cases in which a joint became tuberculous after a trauma, in a patient who showed no other evidence of a tuberculous infection. But the fact that such cases are rare makes it probable that constitutional, genetic, differences in resistance exist between individuals. The same holds true for traumatic carcinoma.

It is evident that the study of the inheritance of constitutional predisposition to a disease must be almost impossible, where infection is so general as in the case of tuberculosis. We can only hope to find instances of the inheritance of predisposition or reversely, of immunity to a bacterial disease in cases where we are dealing with one, or with very few genetic factors, genes, whose influence on the resistance happens to be very marked indeed.

Now, in principle, there are reasons to believe in the possibility of an inheritance of immunity or predisposition for bacterial diseases. In the first place we have those instances, in which closely related varieties or species differ in resistance to a specific bacterial infection. A classical instance is that of the Algerian sheep, which are constitutionally immune to anthrax.

Another, similar instance was met by us in our work with rats. We found that there was a striking lack of uniformity in the practical results of the use of a paratyphus culture as distributed by the State Serum-institute of Holland for exterminating rats. In some parts of Holland the broth-culture was highly effective and very well spoken of, whereas it was almost wholly ineffective in other provinces. It appeared to us that this difference might depend upon the species of rats against which the culture was used. It was discovered by some joint work of the Koloniaal

Instituut and ourselves, that the Norway rat, which is the common rat in most parts of Holland, was practically, if not wholly, absent from parts of Friesland. In these parts *Mus rattus* is the common rat. Whereas *Mus norvegicus* succumbs readily to an ingestion of the broth culture as prepared by the Institute, we found the *Mus rattus* animals immune. Before we started for Java, we tried the pathogenic influence of the culture as furnished to farmers, on some of our cultivated rats of the *Mus rattus* group, on request of our ministry of colonial affairs. The rats were fed on a broth culture of a virulent strain of paratyphoid and bread, at the Serum-institute, and they remained in good health on this diet. The same culture killed practically all *Mus norvegicus* rats in a few days.

To our great regret we have never yet succeeded in obtaining hybrids between the two groups of rats, *norvegicus* and *rattus,* and for this reason the inheritance of this very marked immunity of *Mus rattus,* or in other words predisposition of *Mus norvegicus* can not be studied. We know of no case in the literature, of an investigation of the inheritance of immunity to bacterial disease in animals.

As is well known, Biffen found a case of the inheritance of resistance to rust in wheat, in which the difference between immune and easily infected plants was proved to be due to presence or absence of one single gene. William Orton and Webber have since found almost similar instances in cotton and watermelons.

So far as known to the authors, the following case of the inheritance of immunity, or predisposition for a microbial disease in animals is the first one studied so far.

From Nagasaki, Japan, and Hong Kong, China, we brought along some stock of a very minute domestic mouse. These mice evidently belong to the same group as the commonly imported oriental Waltzing mice. As a matter of fact, our Japanese animals of the second importation produced some waltzing offspring. We used this material for a few series of experiments on the inheritance of weight, one series starting from the only fertile Hong Kong female, and the others from diverse combinations of the Nagasaki strain with large white mice. These white mice are of a pure-bred strain used by T. B. Robertson in his experiments on growth. We produced numerous hybrids, great numbers of F_2 animals, and further we are grading back the hybrids both to the dwarf and to the heavy strain. For our work individual

mice are frequently weighed, and from time to time the whole series is weighed.

In the beginning of January an epidemic started in our mousery. Our mice were at that time housed in approximately seven hundred cages containing several thousand mice, both the size-inheritance and other series of breeding experiments. The cages of all the series were mixed and arranged on shelves in three adjacent rooms. The infection apparently swept through the entire colony, notwithstanding our attempts to limit it to one room. The Japanese mice were distributed over all the stacks in all three rooms, most of them mated to big mice or hybrids of different generations. All these mice fell victim to the epidemic, excepting three which we kept for a little while longer, by taking them into the living house at the beginning of the trouble. To our surprise the white mice of Robertson's strain proved immune. Even where the dead Japanese were partially eaten by their mates, these latter remained in good health.

It is clear that the main circumstance, which made it possible for us to see the clearcut segregation about to be described, was the rapid spread of the epidemic. All the Japanese mice were dead before the virulence of the microorganism was materially altered.

The rapid course of the disease made it possible to distinguish simply between dead and surviving mice. As a rule we found that animals contracting the disease presented the bunched up appearance and walked with the small, prancing steps familiar to students of paratyphoid in small rodents. They would be visibly ill for one, two, or exceptionally three days before death. We do not remember having seen one recover.

Professor Hall, of the department of bacteriology, of the University of California, was kind enough to make a bacteriological examination of the dying animals, and was able to isolate the same staphylococcus from the blood of the heart of four animals.

If we count the proportion of the animals which succumbed to the epidemic, we have to limit our countings to groups which are comparable. Immunity can never be anything but relative, and if we want simply to use the fact of survival as a criterion for immunity we must exclude as far as possible other causes of death. Of these the two main causes are death or illness of the mother, causing starvation of the young, and troubles in parturition.

In our study of the inheritance of immunity to this staphylo-

coccus infection we have therefore limited our counts to animals of the same age-group, that is to mice of at least four weeks old and not yet used for breeding.

At the general weighing of January 4, 1919, no losses were observed among the Japanese mice. Shortly afterward the Japanese started to die off. And at the general weighing of February 14, the last Japanese mouse was found dead.

The data given in this paper are taken from the records of this general weighing of February 14, 1919. They include litters of six kinds, pure Japanese, pure Robertson's whites, F_1 hybrids, F_2 hybrids, mice with one parent F_1 and the other Japanese, and such with one parent F_1 and one large parent.

As noted above all the Japanese left in the mousery died between January 4 and February 14, 59 in all. Of these 23 were in the class of weaned young, not yet breeding.

TABLE I

Litters of F_2 Animals Jan 4	Same Litters on Febr. 14	Litters of F_2 Animals Jan 4	Same Litters on Febr. 14
7	4	1	1
3	2	3	3
6	6	3	3
2	2	6	2
6	6	2	2
5	2	5	4
5	4	4	4
4	3	3	2
2	2	1	1
3	3	3	1
4	3	6	5
5	4	7	6
6	3	3	3
6	4	2	2
3	1	4	0
5	3	Total $\overline{125}$	$\overline{91}$

As to the Robertson large strain, no deaths were observed within this period of six weeks among mice of this age class. A very considerable number of these weaned young were growing up in cages together with Japanese of their age and sex.

Between January 4 and February 14 we lost no F_1 animals after weaning age. Strictly comparable to the other lots were only three litters, which were weaned within the critical six weeks and not yet put to breeding. These litters contained fourteen young. All were living on February 14.

This shows how the immunity to this staphylococcus disease of the large albino strain as opposed to the predisposition to it of the Japanese strain, is completely dominant in the hybrids.

To our great surprise we found that this difference between immunity and predisposition was caused by presence or absence of one single genetic factor. In other words, we found a very clear monofactorial Mendelian segregation in F_2. As we are weighing non-breeding F_2 animals up to a relatively high age, thirty-one litters containing 125 animals fell into this class between the two dates.

Of these 125 animals 91 were living on February 14, and 34 had died. (Theoretical expectation 93.75 : 31.25.) See Table I.

If in reality the "Robertson" mice have one gene, lacking in the Japanese, whose presence protects them against death from this infection, we would expect the hybrids to produce 50 per cent. gametes with and as many without this gene. As the Japanese lack this gene, we would expect 50 per cent. of the young from matings between F_1 and Japanese to be immune, and 50 per cent. to die. Fourteen such litters were available for the test, with 57 animals. Of these 57 on February 14, there were 25 left, 32 having died. (Theoretical expectation equality.) See Table II.

TABLE II

Litters of F₁ × Japanese on Jan. 4	Same Litters on Febr. 14	Litters of F₁ × Japanese on Jan. 4	Same Litters on Febr. 14
6	3	2	2
7	1	2	1
3	1	5	1
5	5	3	0
4	4	5	1
6	4	5	0
2	2	2	0
		Total 57	25

In the same class with the other litters we had sixteen litters of young, each from one F_1 and one "Robertson" parent. This gave us 51 mice in this class. Fifty of these were living February 14, one having died. (Theoretical expectation no deaths.)

As will be seen in nearly every case the number of deaths was slightly greater than expectation. Occasional mice will die even when given the best of care. It is indeed remarkable that not more of these vigorous mice, kept for the most part in company with several of their own sex, got killed fighting. It must

be remembered that these figures for deaths comprise all cases of absence. Mice killed in fights and animals escaped are classed as dead.

The numbers published in this note were collected only after the epidemic had done its worst, and from our weighing records. The epidemic seriously interfered with some of our planned series in our breeding work on weight.

It was planned to start a series of infection-experiments with the isolated staphylococcus strains on families of F_2 animals. It may be possible at some future date to do this, when the material will again be in the right condition for the experiment, that is to say, free of spontaneous infection. At present, however, it is evident that the staphylococcus infection is still in our mousery. The mortality in F_2 families remains high. It is clear that, if we subjected F_2 animals to infection with a pure culture of the staphylococcus, the group of animals would be already a selected group, and the results would be quite misleading.

We have refrained from publishing these data for some time, hoping that we could free our mousery from the infection, so that we could repeat under conditions of a laboratory experiment the immunity tests of F_2 families. There seems no further reason now to withhold the facts such as they are.

As far as we are aware no wholly comparable instance is known so far of a gene whose action has such a definite effect upon the resistance to a bacterial disease in animals. The evidence for the inheritance of a differential susceptibility to transplanted tumors in Japanese and large mice in the work of Tyzzer is scarcely as definite as our case.

In any case, this instance recorded here proves clearly that the presence of a definite pathogenic organism as a factor in a transmittable disease need not be the sole determining cause of the disease. And it shows that the search for heritable factors in the causation of bacterial diseases is neither hopeless nor unscientific. We can only hope that cases such as the one just given will encourage those medical investigators who believe that predisposition is a factor not to be lost sight of in the press of bacteriological and related discoveries.

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Berkeley, Cal.,
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NOTE ON THE PHOTIC SENSITIVITY OF THE CHITONS[1]

1. The remarkable sensory organs discovered by Mosely (1885) in the tegmentum of the shell-valves of certain chitons are structurally of such a nature that in their most highly developed forms they were from the first recognized to be "eyes." Practically nothing has been made known as to the functional values of these organs, which in different genera occur in a great diversity of form, number, and arrangement. It has been shown, however, that the tegmental æsthetes of *Chiton tuberculatus* are indeed photosensitive (Arey and Crozier, 1919). But the shell-eyes are in this genus generally represented by structures of an intermediate degree of complexity. The "eyes" are supposed to have been derived from large, relatively undifferentiated shell receptors (macræsthetes), and seem to reach their highest development in those species of *Schizochiton* and *Tonica* which possess large complex eyes, each surrounded by a pigment cup (*cf.* Plate, 1899; Nowikoff, 1907, 1909); in *Chiton* (at least in some species of this genus) the eyes are "*intrapigmental,*" pigment being contained within the receptor cells, whereas with the "*extrapigmental*" eyes the associated pigment occurs outside

[1] Contributions from the Bermuda Biological Station for Research.

the receptor cells proper, in the integument. It seemed profitable to attempt an analysis of the functional values of the several types of photoreceptive elements to be found in different chitons. Accordingly, in 1918 I made observations on the photic irritability of representatives of several genera available at Bermuda. Pending the collection of more information on this subject, which is necessary for a full discussion of the problem here suggested, I give briefly the net result of these observations.

2. *Ischnochiton purpurascens*—found along the shores of islands in Great Sound, and in bays on the south shore of Bermuda, usually in more or less exposed situations, but commonly a little lower than the lowest reach of the tide (never between tidal limits)—is quite sensitive to light. Individuals about 1 cm. long were frequently obtained on bottles which had been on the bottom long enough to acquire a film of algal growth; the under surfaces of such bottles, and of smooth stones, provided most of my specimens. These animals were photonegative to light of any intensity used—from very weak diffuse light to direct sunlight. This species therefore resembles *I. magdalenensis* (Heath, 1899). It is said that among the *Ischnochitoninæ* there are no shell eyes. However, *Boreochiton*, also of this family, never occurs ''far from the light'' (Pelseneer, 1906, p. 50).

I. purpurascens is an active creeper (Crozier, 1919). It orients very quickly and precisely away from a source of illumination. At night its photic irritability seems decidedly enhanced, as I learned by comparing the rate of orientation of single individuals to lamp light, in a dark room, at different times during the twenty-four hours. (This would appear to be the case with *Chiton tuberculatus* also—*cf.* Arey and Crozier, 1919.)

No evidence was had that *I. purpurascens* is reactive to changes of light intensity.

3. *Acanthochites spiculosus.* Specimens about 14 mm. long were found under stones, somewhat beneath low water level, in Ely's Harbor and at Spanish Point. In these places the water of the open ocean is less modified than within the sounds. The requirements of *Acanthochites* seems in this respect more rigorous than are those of the preceding species, for *A. spiculosus* was not found well within Great Sound. As in the case of *Ischnochiton*, the present species is decidedly photosensitive, and orients precisely away from the light. It moves faster away from a bright light than from a weak one, and comes to rest in the shade. It is strongly thigmotactic, tending to settle in the angles at the

corners of an aquarium, and once in such a situation is difficult to move by light. Negative geotropism is also fairly well pronounced.

If the intensity of light falling on an *Acanthochites* be suddenly increased, the girdle is depressed into contact with the substratum. Local illumination confined to the girdle leads to a local response of the same character. The shell plates seem not to be sensitive in this respect.

As in the case of most Chitons (Sampson, 1895; Crozier, 1919; Arey and Crozier, 1919), the body may be strongly curved to one side, the animal pivoting in a circle of short radius. Photic orientation is often accomplished in this way. The "pivoting" of *Acanthochites* ceases instantly when the creature is shaded; orientation is resumed when the light is increased. Since the girdle does not respond to shading of this part alone, I am led to believe that the shell plates are probably responsible for this type of reaction (as with *Chiton;* Arey and Crozier, 1919).

4. As elsewhere described (Arey and Crozier, 1919), the shell-plates of *Chiton tuberculatus* contain receptors activated by light of constant intensity and by shading.

5. An unidentified species of *Tonica*, about 6 mm. long, commonly obtained in company with *Ischnochiton purpurascens*, was found not to be reactive to shading, nor to increase of illumination; but, like the latter, was decidedly photonegative. This form is not so reactive to light as *Ischnochiton*, however.

6. Plate (1901) considered it possible that the order of evolution of the shell eyes of Chitons was from megalæsthetes to intrapigmental eyes to extrapigmental eyes. In the present series of species, this order would be represented by *Ischnochiton, Chiton,* and *Tonica,* in respective sequence. The shell eyes are of course not the only photoreceptors in these animals; for the girdle the ventral surfaces of the body (Arey and Crozier, 1919), and, possibly, the bilateral larval ocelli (Heath, 1904) are functional in this respect. But the experiments recorded in this paper show that functions of a certain diversity are served by the tegmental photoreceptors of the several species. Little can definitely be said, however, regarding the correlation of structural features with functional performance. It is noteworthy that members of the *Ischnochitoninæ*—a group characterized by the absence of shell "eyes" (*i.e.*, with megalæsthetes and micræsthetes only)— are quite as reactive to photic irritation as are members of *Chiton* proper, where, so long as the tegmentum is uneroded, eyes of the

intrapigmental type are functional; they are also more reactive than *Tonica* is, although in the latter extrapigmental eyes are developed. *Acanthochites,* moreover, likewise with intrapigmental "eyes," is reactive to shading, as in the case of *Chiton,* while *Tonica* is not. We are therefore unable to assign definite types of irritability to the several forms of shell photoreceptors.

The position taken by Nowikoff (1909), on morphological grounds, that these organs are not related in genetic sequence, is not inconsistent with such functional data as I possess. He regards the intra- and extrapigmental eyes as being independently derived from megalæsthete structures. It is possible to consider that the megalæsthetes (or certain of them) are activated by light, and that this kind of irritability is simply retained by eyes of the extrapigmental variety, whereas eyes of the intrapigmental sort are in addition activated by shading. However, the local activation of the girdle (of *Chiton*) by light and by shading makes it necessary to believe that tegmental mircæsthetes (*e.g.,* of the girdle scales) may also be implicated in the photic irritability of the shell-plates. As yet, experimental data for the analysis of this problem is incomplete. The possible significance of the number of shell-eyes present also needs to be investigated.

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THE BIONOMICS OF PORICHTHYS NOTATUS GIRARD

Porichthys notatus is a batrachoidid fish, which is known to
range from southern Alaska to the Gulf of California, and from
depths of at least 62 fathoms to just above the lower low-water
level of the reefs.   During the fall and winter months it inhabits
comparatively deep water, where nothing definite is known con-
cerning its life, beyond the fact, recorded by Dr. and Mrs. Eigen-
mann (1889, p. 132), that it is at least occasionally preyed upon
by rock-cods (*Sebastodes*).   In the late spring and early summer
a shoreward migration apparently takes place (Greene, 1899).
Along the coasts of Lower California and on the mainland shore
of southern California, it is usually found in shallow bays at this
season; at Santa Catalina Island Holder (Holder and Jordan,
1909) has mentioned hearing numbers just off rocky shores.
There is but one record of the occurrence of the species in the
reef-pools south of the vicinity of Pt. Conception in California,
Hilton (1914) having found a specimen in a pool on the reef at
Laguna Beach, California.

From Pt. Conception northward, on the contrary, this species,
while never abundant is by no means rare along the reefs within
tidal limits, during the breeding season.   Here it occupies very
shallow, often sandy pools, either those containing boulders or
those with horizontal crevices in the rocky sides.   It is here a
fish of sluggish and retiring habits, swimming slowly with an
undulating motion; when disturbed it usually seeks shelter, but
sometimes swims off a short distance only, coming to rest and

partially covering itself with sand by a lateral twisting of the body. It is able, by a sudden movement of the body, to inflict a rather painful wound with the opercular spine.

The stomachs of the specimens examined from reef-pools were mostly empty; one contained some sand and an empty snail shell, while another had eaten a crab (*Petrolisthes*), of a species which abounds beneath stones along the fore-shore. Eigenmann (1892) found an anchovy in the stomach of a *Porichthys* from San Diego Bay, and the writer found a sardine (*Sardinia cærulea*) in the stomach of a specimen from San Diego County.

The adult is dull brownish, varying very little in color and not much in shade. The photophores are evident as silvery spots, due to the reflection of external light. There is a whitish translucent spot below the eye, and another behind the pectoral fin, in the position of a large pore in *Batrachus tau*. Owing perhaps to a greater development of black pigment, the males retain more of the dark pattern of the young than the females do. The coloration of specimens 26 mm. long was described in the field as follows. Eight greenish black bars extend from the mediodorsal line to the upper edge of a broad silvery stripe with metallic reflections, which occupies the middle third of the body. The fins are clear, excepting a basal caudal bar, and the two dorsal spines. The head is mottled with dark above, and is silvery on the sides and clear below, excepting the dark ring surrounding each photophore. A conspicuous narrow black streak, located below the eye, branches once or twice posteriorly.

Parental care is generally practised in the Batrachoididæ. *Porichthys notatus*, as noted above, after migrating shoreward during the late spring and early summer, breeds in shallow water, within tidal limits from the region of Pt. Conception northward, where all of the following observations were made. No details of the breeding habits prior to the guarding of the eggs have heretofore been published.   Males with enlarged testes were taken by the writer on several occasions from June 2 to June 15, in no case guarding eggs, and in one instance, on June 20, one was found in company with a female containing matured eggs. A male with ripe testes was found washed up on the beach near the reef of Government Pt., near Pt. Conception, on July 15. The females must leave the pools as soon as, or soon after, the eggs are laid, as none other than the one just mentioned was observed in the tidal zone. As Greene (1899) has already remarked, it is the males which guard the eggs and

young, remaining within a few feet of them even when dis-
turbed.   The eggs are cemented to the roof overlying a shallow
crevice in the rocks or a space beneath a flat boulder.   They
somewhat suggest the familiar egg of the Pacific salmon in color,
and vary in the larger diameter from 4.0 to 6.0 mm.   They are
slightly compressed, as though by pressure against the rock, and
are broadly elliptical in outline.

The young hatch out during the summer.   Jordan and Starks
(1895, p. 840), in discussing the species as found in Puget
Sound, remark "the young fasten themselves to the rocks by
means of a ventral disc which soon disappears."   They mention
further that "the adult remains with the young until they are
quite well matured."   On October 25 the writer found a single
grunting male under a large flat stone in a pool about two feet
square, with numerous young all about 26 mm. long.   Other
young, 22 to 28 mm. long, were caught in a larger pool on Octo-
ber 26.   None has been obtained on the reefs in the winter or
spring; young as small as 23 mm. have been taken by the Scripps
Institution for Biological Research, at La Jolla in depths as
great as forty fathoms.   Except for their proximity to the eggs,
the males show no special habits which might be construed as
definitely protective.

Porichthys is one of three genera of phosphorescent shore-
fishes, the other two being *Anomalops* and *Photoblepharon* of
the East Indian reefs.   In each of these East Indian fishes the
single large light-producing structure is located below the eye
(Steche, 1909), while in *Porichthys* a large number of photo-
phores (in *P. notatus* Greene found an average of about 700)
are developed in connection with the several lateral lines (except
the uppermost, which is only rarely accompanied by a few rudi-
mentary light organs), one photophore being opposite each pore.
The photophores are most abundantly developed on the ventral
surface, and all are oriented downward.   The same condition
holds true in the several other diverse groups of fishes, mostly
pelagic or bathypelagic, in which the power to emit light has
obviously been independently acquired, as well as in certain
other phosphorescent animals, such as the bathybial cephalopods.
This general downward cast of the light of luminescent marine
animals, a point regarded by the writer as of critical significance,
has apparently not been duly considered by any of the authors
who have proposed such varied theories to explain the biological
significance of biophotogenesis.

The histology of the photophores of *Porichthys notatus* has been reported upon in detail by Greene (1899), and ten years earlier by Eigenmann and Eigenmann (1889), and by Test (1889). These organs were found by the writer to be completely and typically developed in young about 25 mm. long (sectioned at Stanford University). Each photophore lies deeply imbedded in the dermis. It consists essentially of a more or less parabolic reflector surrounded by a mass of melanophores, and enclosing the photogenic gland cells, which are richly supplied with blood vessels, but according to Greene not specifically enervated. The light passes downward either directly from these cells, or indirectly from the reflector, through the large lens and the cornea-like epidermal covering of the organ. The ventral aspect of the illuminated fish is striking, the hundreds of dots of light being arranged in a symmetrical pattern.

The light of *Porichthys* has been observed only in the laboratory, where it has been produced as a response to intense electrical or chemical stimulus. Green records but one instance of a slight glow of the photophores being produced by mechanical stimulus. In experimenting with two specimens from rather deep-water, the same author was unable to produce any reaction of the photophores, although he applied stimuli successful in the case of individuals from the reefs. This observation, while of course insufficient, perhaps indicates that the luminescence may be characteristic of the breeding season. In this connection it is also significant to note that Holder's observations (Holder and Jordan, 1909) indicate that the species is of nocturnal habits, its grunting being heard chiefly at night, or in the evening or early morning.

The peculiar humming sound produced by this species during the night, or during the day when disturbed, is another of its remarkable characteristics. The sound (which by some has been called a ''song,'' an expression which seems quite figurative) is produced in the air-bladder, which has a very thick muscular wall, and is enervated by the thickest ramus of each vagus nerve. Upon opening a live specimen the air-bladder was observed by the writer to vibrate rapidly while the fish was grunting, and the sides of the body were felt vibrating at the same time. The abdomen of young about 25 mm. long was also felt vibrating, but the sound produced, if any, was inaudible.

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